

IOWA STATE COLLEGE  
**JOURNAL OF SCIENCE**

*A Quarterly of Research*



VOL. IV  
1929-30

PUBLISHED BY THE  
EDITORIAL BOARD OF THE  
IOWA STATE COLLEGE JOURNAL OF SCIENCE

IOWA STATE COLLEGE

# JOURNAL OF SCIENCE

Published on the first day of October, January, April and July.

---

## EDITORIAL BOARD

EDITOR-IN-CHIEF, R. E. Buchanan.

ASSOCIATE EDITORS: P. E. Brown, Secretary; C. J. Drake, A. H. Fuller,  
I. E. Melhus, E. A. Benbrook, P. Mabel Nelson, V. E. Nelson.

ADVISORY EDITORS: R. E. Buchanan, Chairman; C. J. Drake, E. W. Lind-  
strom, I. E. Melhus, P. Mabel Nelson, O. R. Sweeney.

BUSINESS COMMITTEE: P. E. Brown, Chairman; A. H. Fuller, V. E. Nelson,  
Jay W. Woodrow.

---

All manuscripts submitted for publication should be addressed to R. E. Buchanan, Room 101 Science Building, Iowa State College, Ames, Iowa.

All remittances should be addressed to P. E. Brown, Bus. Mgr., Room 25 Hall of Agriculture, Iowa State College, Ames, Iowa.

Single Copies: One Dollar; Annual Subscription Three Dollars; In Canada, Three Dollars and a Quarter; Foreign, Three Dollars and a Half.

Entered as second class matter at the Post Office, Ames, Iowa.

## TABLE OF CONTENTS

### No. 1, October, 1929

The Quantitative Determination of Formic Acid in the Presence of Acetic Acid by a Conductometric Titration. EDMOND E. MOORE WITH ELLIS I. FULMER .....	1
Some Correlations of Constitution with Sweet Taste in the Furan Series. HENRY GILMAN AND AMIOT P. HEWLETT .....	27
The Preparation of Furfural Diacetate. HENRY GILMAN AND GEORGE F. WRIGHT .....	35
Antiseptic Hypochlorite by Electrolysis. R. L. VAN PUERSEM, B. K. POSPISHUL AND W. D. HARRIS .....	37

### No. 2, January, 1930

Studies on the Insect Fauna of Iowa Prairies. GEORGE O. HENDRICKSON .....	49
Clarification of Milk for American Cheddar Cheese. G. WILSTER .....	181
Experiments on the Physiological Relationships Between the Stomach Infusoria of Ruminants and Their Hosts, With a Bibliography. ELERY R. BECKER, J. A. SCHULZ AND M. A. EMMERSON .....	215
The Pigeon Fly and Pigeon Malaria in Iowa C. J. DRAKE AND R. M. JONES .....	253
Studies of Crowngall, Overgrowths and Hairyrout on Apple Nursery Stock. J. H. MUNCIE AND ROSS F. SUIT .....	263

### No. 3, April, 1930

Crowngall of <i>Rumex crispus</i> L. and <i>Rheum rhaponticum</i> L. J. H. MUNCIE .....	315
The Oxidation of Phenylmagnesium Bromide in the Presence of Tetraethyl Lead. HENRY GILMAN AND J. A. LEERMAKERS .....	323
Phosphorus Assimilation by Certain Soil Molds. P. E. BROWN AND F. B. SMITH .....	325
The Biological Estimation of Glucose. I. A Study of Factors Influencing Changes in H-Ion Concentration. AMY LE VESCONTE WITH J. H. BUCHANAN AND MAX LEVINE .....	331
The Normal Variability of the Sex-Ratio in the Guinea Pig. R. G. SCHOTT AND W. V. LAMBERT .....	343



The Effect of an Excess of Magnesium on the Volume of Gas Liberated Incidental to the Preparation of Some Alkylmagnesium Halides. HENRY GILMAN AND ROBERT E. FOTHERGILL .....	351
The Preparation of Furylacrylic Acid. HENRY GILMAN, R. E. BROWN, G. F. WRIGHT AND A. P. HEWLETT .....	355

#### No. 4, July, 1930

A Monograph of the Protozoa of the Large Intestine of the Horse. TA-SHIH HSIUNG .....	359
Studies on Nitrogen Fixation in Some Iowa Soils. R. H. WALKER AND P. E. BROWN .....	425
The Variation of Hydrogen Ion Concentration with Carbon Dioxide Pressure Above One Atmosphere. I. Colorimetric Measurement. MARJORIE B. MOORE WITH J. H. BUCHANAN .....	431
The Properties of Starch with Relation to Time of Formation of Starch Gels. O. W. CHAPMAN WITH J. H. BUCHANAN .....	441
The Biological Estimation of Glucose. II. The Relation Between Glu- cose Concentration and the pH Change in Media. AMY LE VES- CONTE WITH J. H. BUCHANAN AND MAX LEVINE .....	451
Determination of Organic Acids. II. Determination of Mixtures of Two Fatty Acids by Partition Between Ethyl Ether and Water. C. H. WERKMAN .....	459
The Esterase and Protease of <i>Penicillium roqueforti</i> . N. M. NAYLOR, L. WEISBRODT SMITH, AND HELEN JO COLLINS.....	465



# THE QUANTITATIVE DETERMINATION OF FORMIC ACID IN THE PRESENCE OF ACETIC ACID BY A CONDUCTOMETRIC TITRATION\*

BY EDMOND E. MOORE WITH ELLIS I. FULMER.

*From the laboratory of biophysical chemistry, Iowa State College.*

Accepted for publication June 3, 1929.

## OUTLINE

- I. Introduction.
- II. Review of quantitative methods for the determination of formic acid.
- III. Theoretical discussion of conductometric titration.
- IV. Equipment and procedure.
- V. Experimental results.
- VI. The use of the curves and diagram in analysis.
- VII. Summary.
- VIII. Literature cited.

## I. INTRODUCTION

Quantitative studies of the chemical activities of microorganisms are at present handicapped by the lack of adequate analytical methods. The analytical problem is made complex by the fact that there are present in a fermentation medium a large variety of organic compounds representing a number of homologous series. After the separation of the series the next step is that of separating the members of a given series, for example the aliphatic monocarboxylic acids, the monohydric alcohols, the glycols and the non-volatile acids.

While a voluminous literature is available on the chemical activities of microorganisms, much of the data are qualitative, or quantitative because of special conditions under which only one or at most two members of a given series are assumed to be produced, or only a limited number of the products are considered. Zymology will be truly quantitative only when it is possible to make a complete chemical "flow sheet" of a given organism, on a given substrate under controlled conditions.

The work presented in this paper is a preliminary report on a program of research on the development of adequate methods for the quantitative determination of fermentation products and deals with the two acids, formic and acetic, of the saturated aliphatic mono-basic acid series. Later communications will be concerned with combinations of other acids of this series in mixtures. The general procedure here outlined in some detail will be applicable to other phases of the problem.

\* This communication is based upon a thesis submitted by Edmond E. Moore in partial fulfillment of the requirements for the degree Doctor of Philosophy in chemistry at Iowa State College.

## II. REVIEW OF METHODS FOR THE QUANTITATIVE DETERMINATION OF FORMIC ACID

The following methods have been used for the determination of formic acid in the presence of one or more of the other volatile fatty acids. In each case typical references precede the brief discussion.

*Reduction of mercuric salts.* Auerbach and Puddelman (1910). Auerbach and Zeglin (1922), Benedict and Harrop (1922), Fincke (1913), Franzen and Egger (1911), Franzen and Greve (1910), Holmerg and Lindberg (1923), Oberhauser and Hensinger (1927), Ost and Klein (1908), Pregl (1917), Riesser (1916) and Utkin-Ljubowzoff (1923).

The reduced mercury salt can be weighed or determined iodometrically. The method is accurate but time consuming. The reduction usually requires several hours of heating on a water-bath in the presence of suitable catalysts. The subsequent determination of the quantity of mercurous salt formed also requires considerable time.

*Reduction of platinum chloride to metallic platinum.* Bacon (1911) has used this method for the determination of small quantities of formic acid.

*Reduction of iodates.* Cuny (1911). This method is similar to those used in the reduction of mercuric salts. First, the sample is refluxed with iodic acid, the solution having been made strongly acid with sulfuric acid, then the iodine is liberated, distilled off, and titrated with sodium thiosulfate. The method requires as much time as that using the reduction of mercuric salts.

*Reduction of potassium permanganate.* Fouchet (1912), Grossman and Aufrecht (1906), Oberhauser and Hensinger (1927), Ost and Klein (1908), Whittier (1923).

If the temperature, concentration and time are properly regulated, the formates in a solution which has been made alkaline with sodium carbonate will be oxidized by potassium permanganate, while the salts of the other acids will not be affected. This method has been modified many times, but has never become popular.

*The reduction of bromine.* Joseph (1912). Oberhauser and Hensinger (1927). The first method was shown to give unsatisfactory results. The second is claimed by the authors to be better. The formic acid is treated with bromine in potassium bromide, an excess of standard sodium dihydrogen arsenite is added and the solution titrated with bromine in potassium bromide, using indigo carmenstyphinic acid 2:1 as indicator. The method is time consuming.

*The reduction of chromic acid.* MacNair (1887), Nicloux (1897), Ost and Klein (1908), Tsinopinas (1917).

The method consists in boiling the sample with potassium dichromate and sulfuric acid and titrating back with potassium iodide and sodium thiosulfate. It has the same objections as other reduction methods.



*Decomposition by concentrated sulfuric acid and measurement of the carbon monoxide evolved.* Ost and Klein (1908), Wegener (1903). The method is applicable only to concentrated solutions.

*Reduction by metallic magnesium.* Fenton and Sisson (1907). The method is not accurate enough for quantitative work.

*Displacement of the acetate by the formate radical.* Heermann (1915). The formate and acetic acids can be converted to the sodium salts and weighed, then by treatment with formic acid the whole can be changed to sodium formate, and again weighed. The first weight gives the sum of the weights of sodium formate and sodium acetate, and the second weight gives the weight of the sodium formate plus that of the sodium acetate expressed as sodium formate. From these data the weights of the formic and acetic acids originally present can be calculated. The method has been criticized by Laufmann (1915), who reported that all the acetic acid was not expelled even after several evaporations with formic acid. It would not be applicable in the presence of other acids than acetic.

*Micro-analytical methods.* Wohack (1921). These are based on the reduction of mercuric salts and differ from those given above mainly in the quantities used.

*Separation by distillation depending on the various rates of distillation of different acids.* The method was first proposed by Liebig (1849). Duclaux (1895) devised a method of analysis which was based on this principle. He distilled 110 c.c. of a solution containing 1 to 2 per cent of acid. 100 c.c. of the distillate were collected in ten fractions of ten c.c. each. The ratios between the sets of values obtained by titrating these fractions and those obtained on titrating the whole sample are known as Duclaux's constants. These constants serve to identify any particular acid. By assuming that each acid in a mixture behaves as if it were there alone Duclaux extended his method to the analysis of mixtures. This procedure has been used by many investigators, but has been subject to much criticism, especially by Richmond (1895), and Upson, Plum and Scott (1917).

*Steam distillation.* Dyer (1917) introduced a method of steam distillation. He aimed at keeping the volume approximately constant by introducing steam. He regulated the source of heat and the supply of steam so that the level of the liquid in the flask did not alter appreciably. This method lessens one of the more serious errors in the Duclaux procedure in that during the distillation of an aqueous solution, unless the solute has the same rate of volatilization as the solvent, the concentration of the latter will change. Richmond (1895) found that, in the case of formic, and to a less extent of acetic acid, the rate of distillation is influenced by the concentration of the solutes. In the case of an ordinary distillation in which the substance in solution distills at a slower rate than the solvent, the solution will become more concentrated with the consequent alteration of constants. In an unknown mixture a correction cannot be applied with certainty in such cases. Variations in concentration, with consequent alteration of constants, will be reduced if the distillation is carried out at constant volume. The limits of variation can be reduced still further if rela-



tively large volumes of solution are distilled compared with the volume of distillate collected.

The following table gives the data upon which Dyer's method is based. It gives the comparative percentages of acid which distill over into the first 100 c.c. fraction of distillate from a constant volume of 150 c.c.

Table 1. Percent of acids distilling according to Dyer (1917)

Formic	Acetic	Propionic	n-Butyric
17.89	30.75	52.67	69.88

The volatility with steam increases with the addition of  $\text{CH}_2$  groups. Dyer repeated the above, titrating 10 c.c. fractions in each case, and found that the percentage figure for the first 10 c.c. fraction is the distilling constant for each acid, "or is that percentage of the residual acid which distills over in each successive 10 c.c. fraction." He was able to show from theoretical calculations based upon the above that the percentage of any single acid distilled over at any time is a simple logarithmic function of the cubic centimeters of distillate obtained. These deductions he checked experimentally. He then was able to represent on a logarithmic chart the percentage of acid distilled over for any given amount of distillate.

The identification of a single acid by this method is carried out as follows: The total acid present is titrated with 0.1 N alkali, the distillation is then so arranged that the amount of pure acid contained in the total 150 c.c. volume in the distilling flask is about 0.5 c.c. For distillation the acid is liberated with an equivalent amount of 0.1 N or 0.2 N sulfuric acid. Any convenient amount of distillate, for example 100 c.c., is collected. This is titrated with 0.1 N alkali and the value obtained divided by that secured in the original titration. The result will be the distilling constant of the acid for the first 100 c.c. fraction of distillate. The nature of the acid distilled can be found by comparing the value obtained with the logarithmic chart.

Mixtures of acids upon distillation give curved instead of straight lines when represented on the chart. With neighboring acids these curved lines will be very flat, while the further removed from each other the acids are in their relative positions on the chart, the greater will be the bulge in the curve. In the case of formic and acetic acids, since the distilling constant of each pure acid is known, and the distilling constant of the mixture is obtained, one can calculate in what proportions they are mixed. The use of the method with mixture of acids which are not neighboring is questioned. Dyer states, "The lowest acid of the series in the mixture will be identified with certainty since it will eventually reach a point where it will begin to parallel some straight line on the chart which represents that acid. This same point indicates where the other acid of the mixture has run out and reference to a table given will indicate with a fair degree of certainty the nature of this accompanying acid." Reilly and Hickenbottom (1919) do not agree with the above; they state, "By distilling an aqueous solution of a mixture of two acids having different rates of volatilization, it becomes evident that as the distillation proceeds, the solution in the flask will become relatively richer in the less volatile acid,

so that the solution gradually changes in composition with a corresponding change in vapor. It is obvious, therefore, that although a consideration of the logarithmic and other constants will give an accurate idea of the composition of the vapor at any given time, it cannot be applied with certainty to the composition of the solution distilled. The method suggested by Dyer of taking the point at which the logarithmic curve of the mixture becomes parallel to that of the less volatile component is therefore open to a very wide error, and can be only approximate."

Donker (1926) suggested a method of adjusting the hydrogen ion concentration so that the formic acid would distill over while the acetic acid would not. Because of the small differences in dissociation constants in these two acids the method would appear to be difficult, if at all practical.

Variations in solubility between adjacent members of the fatty acid series or corresponding derivatives are usually small and sharp separations by the solubility method are hard to obtain.

We were unable to find any reference to methods for the determination of formic acid by conductivity methods. Kolthoff (1923) gives a method for determining the total volatile fatty acids by a conductometric method. He titrated the mixture with mercuric perchlorate, and obtained a sharp change, or break, in the conductivity curve due to the formation of the slightly dissociated mercuric salts. He does not propose a method for the determination of the individual members of the series.

### III. GENERAL DISCUSSION OF CONDUCTOMETRIC TITRATION.

For a summary of the theory and applications of conductometric titrations the reader is referred to a monograph on that subject by Kolthoff (1923). The conductivity of an acid or of a mixture of acids, at a given concentration, is a function of the temperature, the viscosity of the medium, the degree of dissociation of the solutes, and the mobility of the ions. By using dilute solutions at constant temperature and employing acids of a similar nature, such as those used in these studies, the changes due to variation in viscosity are reduced to a minimum. In table 2 are given data on the specific conductivity of a strong acid, hydrochloric, and a weak acid, formic acid as affected by the addition of a strong base, potassium hydroxide. The data are plotted in figures 1 and 2.

It is seen that with hydrochloric acid there is a marked decrease in the specific conductivity to the neutralization point at which the specific conductivity increases rapidly. Hydrochloric acid of the strength used will be very largely dissociated and therefore the conductivity will be high due to the large number of highly mobile hydrogen ions present. As potassium hydroxide is added the hydrogen ions are replaced by the less mobile potassium ions until neutralization is reached. The addition of more potassium hydroxide now causes an increase in conductivity and adds some of the highly mobile hydroxyl ions. Eastman (1925) shows mathematically that this end-point should check the true neutralization point within a few thousandths of one per cent.

If in place of hydrochloric acid we use a weak acid, for example formic, and neutralize with potassium hydroxide, at first the conductivity



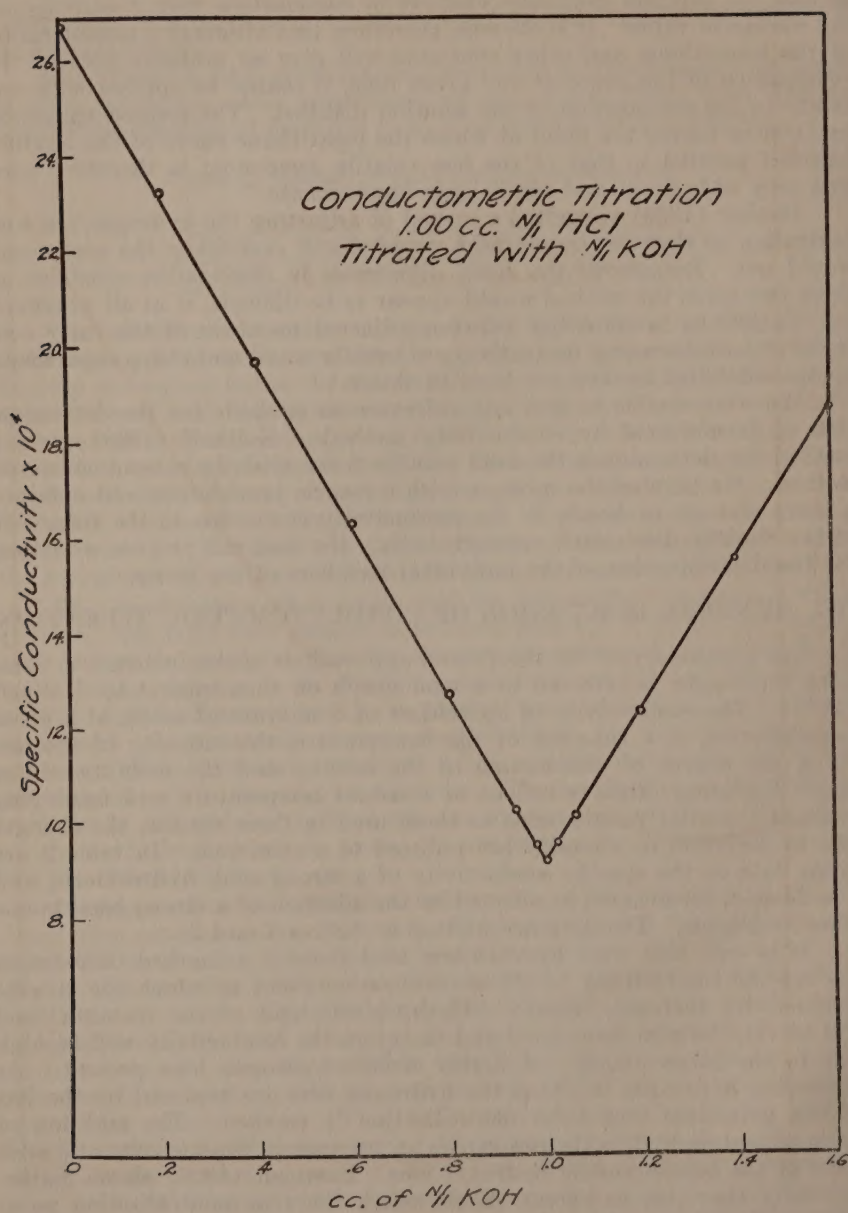


Fig. 1



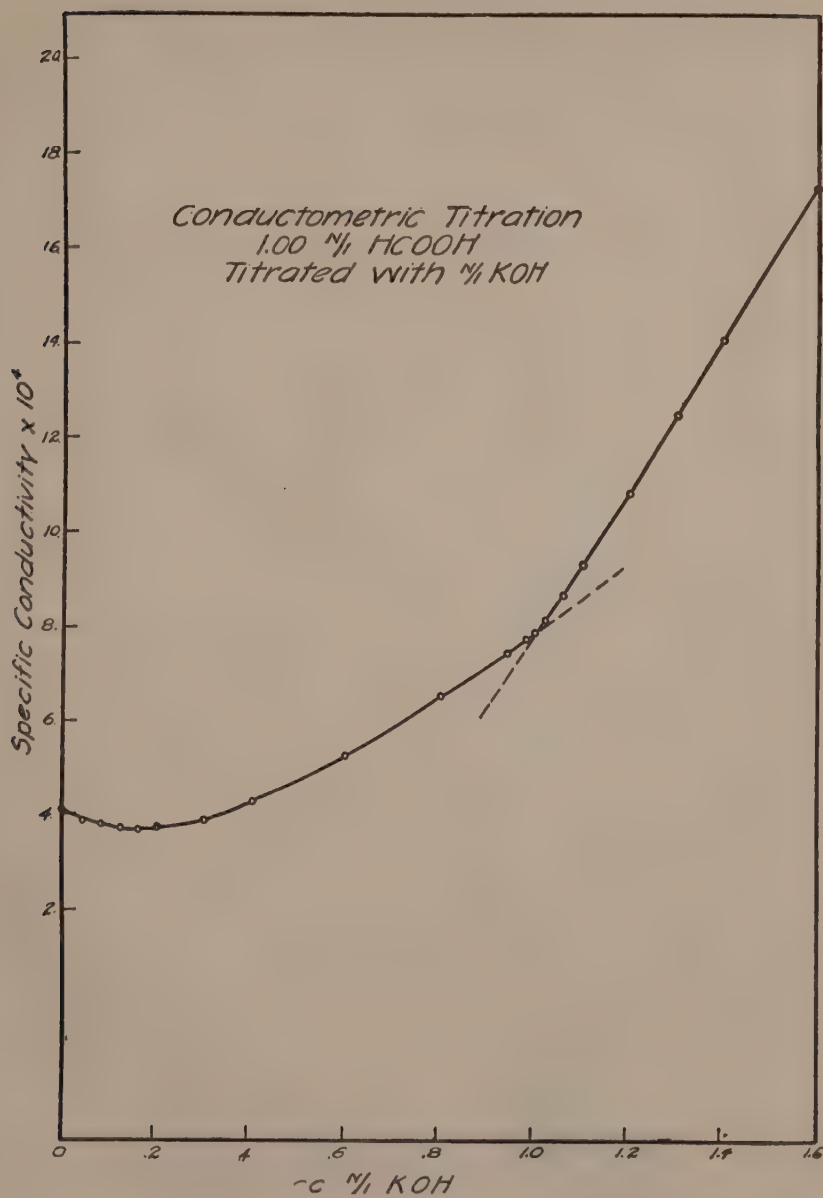


Fig. 2

TABLE 2. Titration of 1 c.c. of N HCl and of 1c.c. of N HCOOH in 156 c.c. of solution (0.0641 N) with N KOH

HCl		HCOOH	
c.c. Alkali	Specific conductiv- ity $\times 10^4$	c.c. Alkali	Specific conductiv- ity $\times 10^4$
0.00	26.77	0.00	4.054
0.20	23.28	0.04	3.861
0.40	19.68	0.08	3.728
0.60	16.18	0.12	3.640
0.80	12.61	0.16	3.612
0.94	10.19	0.20	3.624
0.98	9.47	0.30	3.846
1.00	9.29	0.40	4.214
1.02	9.51	0.60	5.246
1.06	10.01	0.80	6.485
1.10	10.59	0.94	7.411
1.20	12.23	0.98	7.692
1.40	15.40	1.00	7.848
1.60	18.59	1.02	8.088
1.80	21.88	1.06	8.677
		1.10	9.264
		1.20	10.820
		1.30	12.440
		1.40	14.060
		1.80	20.440
		1.60	17.290

decreases as in the case of the strong acid, but it begins to increase before all of the acid is neutralized, and at the neutralization point the conductivity increase becomes still greater. The formic acid is not as highly ionized as the hydrochloric and thus the initial conductivity is less. As potassium hydroxide is added, potassium formate, which is highly ionized, is formed. This cuts down the ionization of the formic acid. A point is soon reached where the decrease in conductivity due to the decrease in hydrogen ions is less than the increase in conductivity due to the increase in number of potassium and formate ions. The location of this point depends upon the concentration of the acid. In a very dilute solution it would approach the neutralization point. This minimum is not a definite sharp "break" as in the case of a strong acid and strong base, but a rounded "hump", which makes it unsuited for use in analytical work. When the minimum part of the curve has been passed the conductivity continues to increase evenly up to the neutralization point, where there is a "break" in the curve, the conductivity increasing more rapidly but again evenly because of the increasing numbers of the more mobile hydroxyl ions, as in the case of the strong acid and strong base.

In table 3 are given data for the conductometric titration of formic acid, acetic acid and a mixture of equal amounts of these acids with the weak base ammonium hydroxide. The data are plotted on figure 3. The large excess of acid present at first hinders the hydrolysis of the salt. The conductivity passes thru a minimum and then increases as in the above case. In the vicinity of the end-point hydrolysis becomes large and the conductivity increases less rapidly. When an excess of the weak base has been added the hydrolysis will be suppressed and there will be no further measurable change in conductivity.

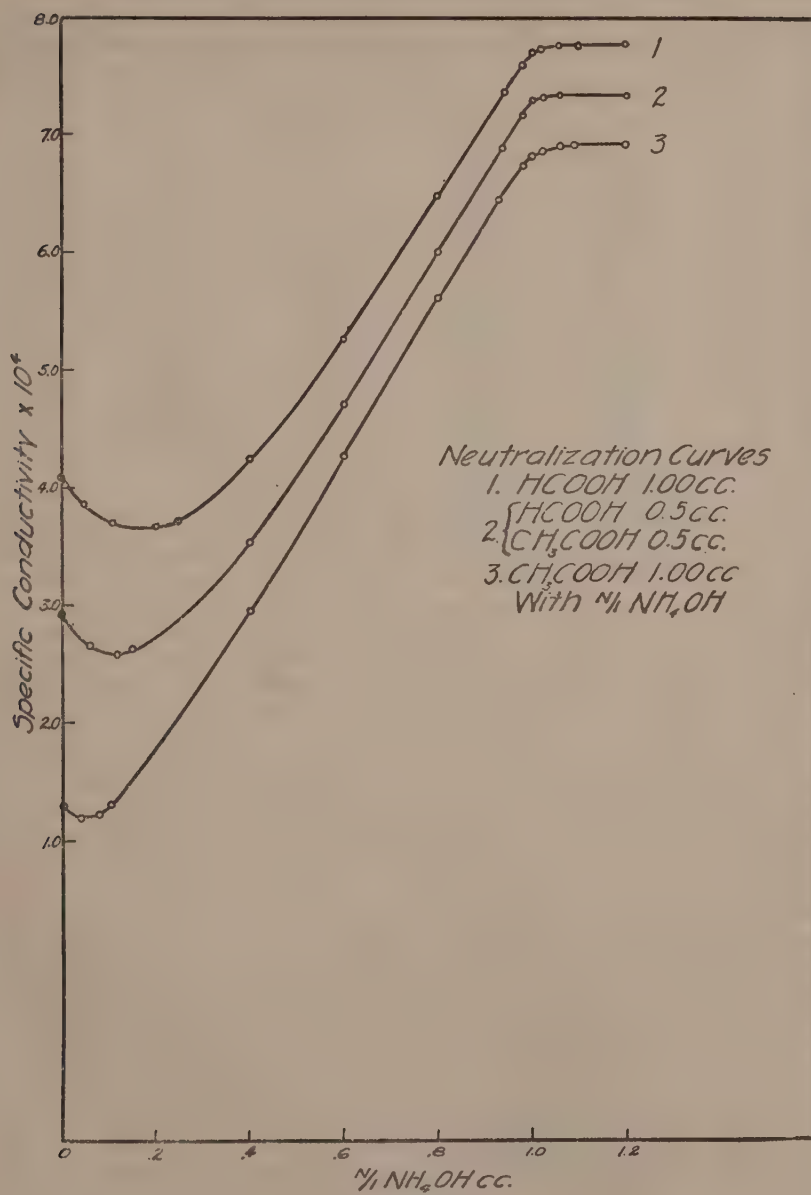


Fig. 3



TABLE 3. Titration of  $\text{HCOOH}$ ,  $\text{CH}_3\text{COOH}$ , and a mixture of the acids with  $\text{NH}_4\text{OH}$ , (1 N) total concentration of acids = 0.00641 N

1 c.c. $\text{HCOOH}$ diluted to 156 c.c.		1 c.c. $\text{CH}_3\text{COOH}$ diluted to 156 c.c.		0.50 c.c. $\text{HCOOH}$ , 0.50 c.c. $\text{CH}_3\text{COOH}$ diluted to 156 c.c.	
c.c. Alkali	Sp. Cond. $\times 10^4$	c.c. Alkali	Sp. cond. $\times 10^4$	c.c. Alkali	Sp. cond. $\times 10^4$
0.00	4.057	0.00	1.281	0.00	2.898
0.05	3.830	0.04	1.174	0.06	2.655
0.12	3.657	0.08	1.212	0.12	2.562
0.20	3.643	0.10	1.287	0.16	2.587
0.25	3.678	0.40	2.942	0.40	3.503
0.40	4.222	0.60	4.248	0.60	4.717
0.60	5.254	0.80	5.592	0.80	5.999
0.80	6.469	0.94	6.457	0.94	6.868
0.94	7.377	0.98	6.739	0.98	7.188
0.98	7.608	1.00	6.838	1.00	7.269
1.00	7.703	1.02	6.853	1.02	7.319
1.02	7.731	1.06	6.923	1.06	7.350
1.06	7.759	1.08	6.923	1.08	7.350
1.08	7.771	1.10	6.923	1.10	7.350
1.10	7.771	1.20	6.923	1.20	7.350
1.20	7.771				

The reactions from the beginning of the titration until hydrolysis begins to take place are the same as in the case of a weak acid and a strong base. Near the neutral point the highly ionized ammonium formate is hydrolyzed to formic acid and ammonium hydroxide, both of which are but slightly ionized in the presence of ammonium formate, thus slowing up the increase in conductivity. After the end-point has been passed the addition of more ammonium hydroxide will cause practically no change in conductivity, as ammonium hydroxide is but slightly ionized in the presence of its salts. In the concentrations used in this work the increase in conductivity due to the presence of more base is balanced by the decrease due to the dilution of salt solution. The end-points in the last two cases may differ considerably from the true equivalence points. Where such differences affect the method outlined they will be discussed later in this paper. For a complete discussion of the subject one should refer to Kolthoff (1923) and Eastman (1925).

It is possible to determine a strong acid and a weak acid or two weak acids of very different dissociation constants, in the same solution by a single titration with an alkali. The stronger acid is neutralized first, then the weaker, so that there are breaks in the curve corresponding to the amount of each acid present. Kolthoff (1923) shows that this method cannot be used if the ratio of the dissociation constants of the two acids is less than 1000 or if the mobilities of the anions of the two acids are nearly the same. The dissociation constants of the first members of the volatile fatty acids are so near together that an analysis of a mixture by the usual conductivity titration is not feasible.

As stated above, if a weak acid is titrated with a weak base, a point is reached, soon after the true neutralization point, beyond which there is no further change in conductivity on the addition of more base. This point is about six percent higher than the true neutral point for the volatile fatty acids in the concentrations used in this work. A correction fac-

tor has been determined to take care of this difference. Although the usual conductometric methods will not apply to the acids mentioned, the authors propose a method based on the total changes in specific conductivity on titration with ammonium hydroxide.

#### IV. EQUIPMENT AND PROCEDURE

##### PREPARATION OF SOLUTIONS

The hydrochloric acid was standardized by precipitation as  $\text{AgCl}$ .

The potassium hydroxide was standardized by titrating against the standard hydrochloric acid, using methyl orange as the indicator.

The volatile fatty acids used were standardized by titrating against the standard potassium hydroxide, using phenolphthalein as the indicator. The solutions of volatile fatty acids were made from samples which were prepared by taking the purest acids available and fractionally distilling.

The ammonium hydroxide was standardized by titrating against the standard hydrochloric acid, using methyl orange as the indicator. The reference fatty acids and the ammonium hydroxide all had a normality of  $1.00 \pm 0.005$ . As a further check on the concentrations, the volatile fatty acids were titrated against the ammonium hydroxide potentiometrically and found to be equivalent. The specific conductivities of the acids compared very favorably with values in the literature and can be assumed to be well within the limits of purity demanded by the work here outlined.

##### APPARATUS

The thermostat maintained a temperature of  $25^\circ \pm 0.05$ . As the mobilities of the ions increase from 1.5% per degree in the case of hydrogen to 2.7% in the case of carbonate ion, close temperature regulation is necessary.

A Kohlrausch bridge (Leeds and Northrup 4258) was used. This bridge has a 470 centimeter wire of about 7ohms resistance, which is divided into 1000 divisions with half divisions indicated. Our readings were accurate, that is reproducible, to 0.25 divisions. The two plug controlled end coils were each adjusted to 4.5 times the resistance of the slide wire, and were accurate to 0.1 percent.

A direct current cannot be used in the determination of conductivity because electrolysis would occur and the products set free at the electrodes would set up a back E.M.F. and alter the resistance of the liquid, by interposing a layer of gas between the liquids and the electrodes; these effects are included in the term polarization. To avoid this an alternating current is used. Reilly, Rae and Wheeler (1925) give the following as the requirements which must be fulfilled if the current is to be satisfactory for this work:

- (1) The alternations should be sufficiently rapid and the quantity of electricity passing at each alternation should be small so that no appreciable chemical change occurs.

- (2) The quantity of electricity passing in one direction should be exactly equal to that passing in the other—if a small excess passes one way this will produce exactly the same electrode effects as a direct current of the same magnitude.

(3) The source of alternating current should give a wave of pure sine form. If this condition is not fulfilled and harmonics or overtones are present it is difficult to get a good minimum in the telephone.

Taylor and Acree (1916) have shown that with platinized electrodes appreciable differences in the value of the apparent resistance may be produced by a change in frequency of the measuring current, so that the point of balance for the fundamental frequency will not be the same as that of the overtones, with the result that a good sound minimum cannot be obtained.

The induction coil was used by Kohlrausch and Holborn (1898). The best results were obtained with small coils having few turns of wire, so that the total quantity of electricity passing at each pulse was small: the moving parts of the coil being small and light, a high note was obtained to which the ordinary telephone and ear are sensitive. This type of instrument was found to be unsatisfactory in our work; it cannot be used when accuracy greater than 0.2% is required, since the current departs very much from the ideal requirements. Oscillograms obtained from induction coils (Taylor and Acree, 1916) contain a number of harmonics and when the areas of the positive and negative loops are measured it is found that a considerable unidirectional current is present. Since the sum of the two currents in opposite directions is not zero, polarization must occur.

The microphone hummer (Leeds and Northrup No. 9856)\* gave fairly satisfactory results in this work. The frequency is constant since it is maintained by a 1000 cycle tuning fork. This fork actuates the microphone and the current thru the microphone also passes thru a transformer, from the secondary of which the magnet that keeps the fork in vibration is supplied. An extra subdivided winding on the transformer has a number of terminals on the top of the case from which current at different voltages but the same frequency may be obtained. The wave form of the microphone hummer has a number of harmonics which may interfere with the precision of setting the bridge. This difficulty is somewhat lessened if a telephone receiver is used with its resonance point adjusted to the frequency of the microphone hummer. (Leeds and Northrup Catalog 10.) The hummer was kept in a covered box, which had a layer of cotton on the bottom and was lined with cloth to make it practically sound proof. All of the determinations which appear in this paper were made originally using this apparatus.

The two important types of detectors in use are the alternating current galvanometer and the telephone receiver. The former has several advantages over the latter. It is necessary to keep the current on but a short time in finding the balance point, thus decreasing the chances of heating and polarization. Absolute silence is not necessary and the direction of the deflection gives at once the side of the bridge which has the larger resistance. Any creeping of the pointer after balance shows the presence of heating or polarization. For low commercial frequencies the galvanometer is preferred. For higher frequencies, especially 1000 cycles per second, the telephone receiver is twenty times as sensitive as the galvanometer.

\*Mr. R. L. Foster has checked some of our measurements using a Vreeland oscillator and has found the equipment described to be sufficiently precise for the purpose of the particular method here outlined.



This high sensitivity is due to the characteristics of the human ear combined with those of the receiver. The sensitiveness of the telephone is so great that it is eminently satisfactory with a tunable telephone receiver.

To avoid or remedy stray fields, induction effects, etc., it is necessary to surround certain parts of the apparatus with earthed iron cases; for an excellent discussion of these troubles the reader is referred to Reilley, Rae and Wheeler (1925).

The conductivity cell used was made from a 250 c.c. pyrex Erlenmeyer flask by sealing two glass tubes, each having an electrode, into the sides. To increase the rigidity of the apparatus, thin pieces of glass rod were used to connect the four corresponding corners of the two electrodes. The electrodes were made of heavy platinum pieces about one-half inch square and about one inch apart. A clean platinum electrode gives a poor null point due to polarization: a considerable range of silence is obtained and the notes heard on the two sides of the minimum are not of the same tone. Polarization is decreased by platinizing the electrodes. Thick layers of platinum may cause changes in the solution owing to catalytic action or selective adsorption, and by making it difficult to wash out previously used solutions. By using a three percent solution of chlorplatinic acid and reversing the poles every half minute for eight minutes a very satisfactory deposit was secured. This was freed from occluded gases by electrolyzing a dilute solution of sulfuric acid in the cell for half an hour, introducing a third platinum electrode as the anode and having the two electrodes of the conductivity apparatus act as joint cathodes. The cell was hung loosely on a burette clamp in such a way that it could be shaken without removing it from the support. The level of the water in the thermostat should be above the level of the liquid in the cell.

A 2 c.c. burette, graduated to 0.02 c.c. and readable to 0.01 c.c., was used. This had a long tip, which was drawn out at the end so that it was possible to touch it to the surface of the solution in the cell after each addition of reagent; this insured that the reading on the burette really showed the quantity of reagent which had been added to the cell. There was negligible error due to diffusion from the finely drawn out tip, which was in contact with the cell liquid only an instant.

It was found advisable to leave the conductivity cell in the bath during the run, the burette was taken from its stand and the desired amount of reagent run into the cell. The tip of the burette was allowed to touch the surface of the liquid in the cell, the burette was replaced on its stand, the cell was shaken and the bridge reading taken. The new equilibrium is reached quickly. It is not necessary to shake the cell violently or for a long time. A few twists are enough. Neither is it necessary to repeat the shaking and reading to be certain that equilibrium is reached.

#### CELL CONSTANT

The cell constant was determined by using a N/50 KC1 solution at 25° C. Landoldt Börnstein (1923) give 138.65 as the value of the equivalent conductivity of this solution. The dilution water which is used to make up this standard KC1 solution should be the same as that used to dilute the solutions to be titrated. The cell constant for the cell used in the experiments outlined was 0.2709, that is, the conductivities obtained

by the use of the cell are in each instance multiplied by 0.2709 to give the specific conductivity, i. e., the conductivity of one centimeter cube of solution.

The cell should be checked occasionally against a standard KCl solution. If used carefully it will stay constant for a long time in the type of solutions used in these analyses. The constant did not change during the runs made in this work. The electrodes were occasionally saturated with hydrogen by making them the negative poles in a dilute sulfuric acid solution and connecting to two dry cells. This operation appeared to aid in keeping the null point sharp, but did not change the cell constant.

The glass used in conductivity work should be fairly old, as new glass is much more soluble than old.

#### PROCEDURE

155 c.c. of water of a specific resistance of about  $2 \times 10^5$  ohms were used. This is about the quality of water which is obtained upon distillation from pyrex glass apparatus. Quantitative studies showed that the exact value of the specific conductivity of the water used need not be known in this work, nor is it necessary for other workers to use water of the same specific conductivity as that which we used. In fact, a water of no higher conductivity than would be used by any reasonably careful worker, does not cause a sufficient error to vitiate the results.

The desired amount of normal acid was placed in the conductivity flask, to which were added 155 c.c. of dilution water from a calibrated narrow mouth flask. This gave a solution from 0.0065 to 0.013 normal in acid. When the solution is titrated with normal alkali the effect of dilution upon the conductivity is small. Another reason for using such a large cell is the rather low concentration of acids in the distillate of some of our fermentation mixtures.

To run a complete curve, the bridge reading (A) was taken after each addition of alkali, from tables of  $\frac{A}{1000-A}$ , the specific conductivity,

$$S = \frac{A}{1000-A} \cdot \frac{K}{R}$$

$$K = 2.709 \times 10^{-1} = \text{cell constant}$$

R = Standard resistance

S = Specific conductivity

In the ordinary analysis it is not necessary to take many points before the end-point. In fact, it is not advisable to do so as normal ammonium hydroxide loses strength rather quickly when exposed to the air. The initial reading should be accurately determined, as it is from this reading that the specific conductivity of the acids is calculated. Usually the operator will know the total acid concentration from a previous titration so the ammonium hydroxide can be run in rather quickly to this point, then

added 0.05 c.c. at a time until two readings check. This constant reading is used to calculate the specific conductivity of the salt. In case the operator does not know the total acid concentration it is necessary to run in the alkali about 0.02 c.c. at a time until the end-point is nearly reached and then 0.01 c.c. at a time until the bridge readings become constant. The end-point may be obtained in either of two ways. The values obtained may be plotted, specific conductivities against cubic centimeters of alkali added, then by extending the ascending and horizontal lines a point of intersection (see figure 4) will be obtained, which gives the number of cubic centimeters of normal acid. The extended lines referred to above are shown by the broken lines, whose intersection gives the end-point. This method has several disadvantages. First, it is time consuming, second, it is easy to make a sufficient error in drawing the lines to affect the result by several percent, and lastly, the end-point obtained, even if the work has been carefully carried out, is not the true equivalence point. (Eastman 1925.) A second method gives more satisfactory results and requires much less time. If the total number of cubic centimeters of normal ammonium hydroxide which must be added before the conductivity becomes constant is divided by the factor 1.06 the result gives the number of cubic centimeters of normal acid originally present in the solution. This value, 1.06 was obtained by running a number of neutralization curves on various acid solutions of known composition and noting the quantity of normal ammonium hydroxide which must be added before the conductivity became constant. This value, 1.06, is applicable only in the range of concentrations with which we are working. The relationship between equivalence and the quantity of ammonium hydroxide which must be added before the conductivity becomes constant was discussed before in the section on theory.

#### FACTORS INFLUENCING ACCURACY OF DATA

The specific conductivity of a solution can be easily determined with a degree of accuracy greater than is necessary for quantitative work here outlined. The errors, other than those incidental to any quantitative procedure, are due to instability of reagents and to the presence of substances in the solution which affect the conductivity other than those substances being analyzed. We were able to check results repeatedly over intervals of several months using the same standard normal ammonium hydroxide. This was made up in quantities of several liters, and was kept stoppered at all times except when it was necessary to allow some air to enter so that the alkali could run out. The solution became somewhat clouded, but its strength did not change enough to affect the results.

The specific conductivity when the alkali has been added to a constant value includes the dilution effect due to the addition of the water with the alkali. Evidently the particular final values given in our tables apply only for the use of 1 N alkali. If weaker concentrations were used the values would be less than those given above. The relatively strong alkali was used in order to minimize this dilution effect.

It was previously noted that the cell constant was derived in such a way as to correct for the particular water used, that is, for water distilled from pyrex. Water of the above quality was kept for a month in a tin

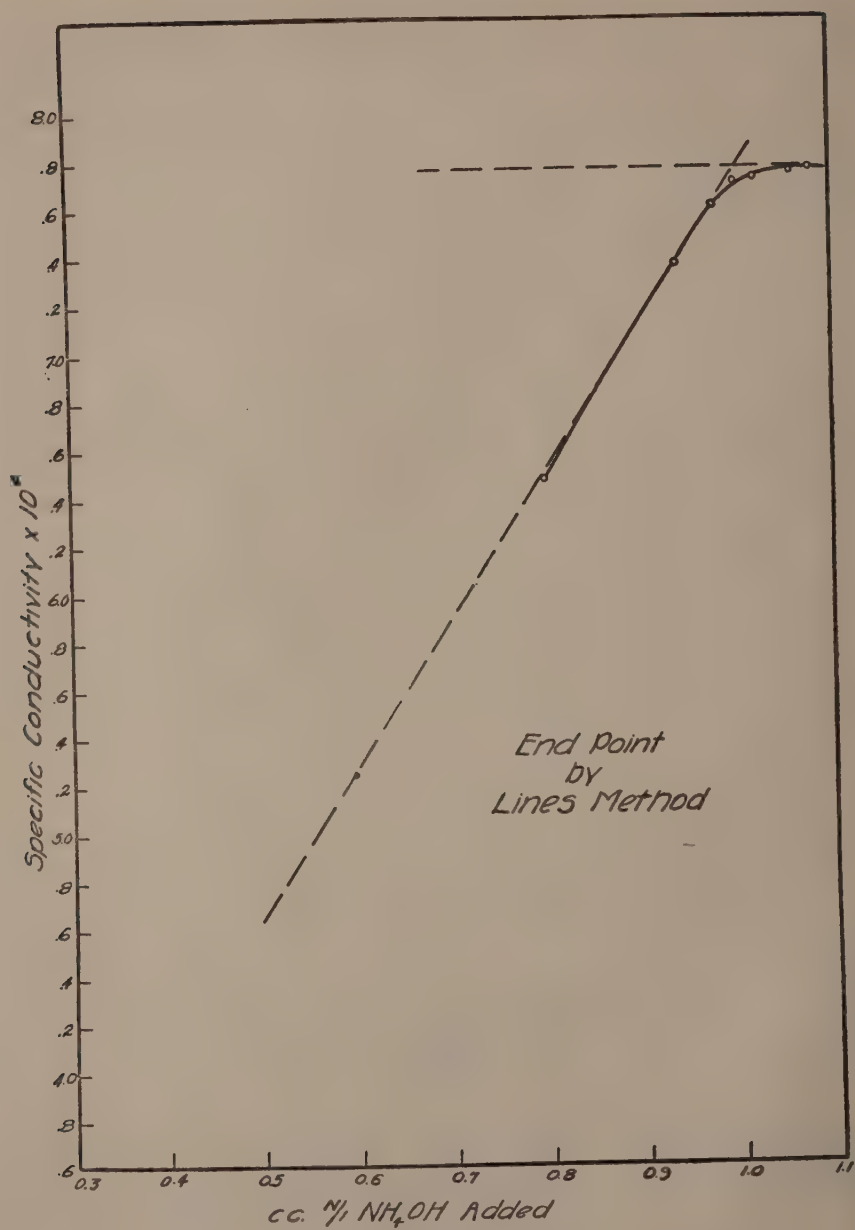


Fig. 4



container with a loose cover. During this time there was not sufficient change in the specific conductivity to affect results to an appreciable degree with the equipment used. Water that has been exposed to the air will produce an appreciable error. However, if ordinary care is used in following the procedure errors will not be great enough to affect analytical results.

## V. EXPERIMENTAL RESULTS

In Table 3 were given data for the conductometric titration of formic acid, acetic acid, and a mixture of the two acids, at 0.00641 N. Later it was proposed to use the total change in specific conductivity on the addition of 1 N ammonium hydroxide as a basis for analysis. In Table 4 are found data of this type for mixtures of the two acids at various concentrations from 0.00641 N—0.01274 N. The data are plotted in Figures 5 and 6.

The data from Table 4 are likewise plotted on the three way Diagram 1. The vertical planes A B C D, E F G H, I J K L, M N O P, and Q R S T represent as ordinates the total changes in specific conductivity and as abscissae the percent of total acid as acetic acid in the mixture of the formic and acetic acids. These planes represent the same curves as shown in Figure 5. The concentrations for these planes are 1.00, 1.25, 1.50, 1.75 and 2.00 or N acid added to 155 c.c. of water representing 0.00641 N, 0.00800 N, 0.00959 N, 0.01116 N and 0.01274 N, respectively. Each profile

TABLE 4. Total change in specific conductivity on titration of mixtures of formic and acetic acids with 1 N ammonium hydroxide

c.c. of N acid added to 155 c.c. water		Pct. of total normality		Normal- ity	Total change in sp. cond. $\times 10^4$
CH <sub>3</sub> COOH	HCOOH	CH <sub>3</sub> COOH	HCOOH		
2.00	0.00	100	0.00	0.01274	11.273
1.50	0.50	75.0	25.00	"	10.472
1.00	1.00	50.0	50.0	"	9.883
0.50	1.50	25.0	75.0	"	9.427
0.00	2.00	0.00	100	"	9.030
1.75	0.00	100	0.00	0.01116	10.059
1.25	0.50	71.42	28.58	"	9.027
0.85	0.90	49.76	50.24	"	8.496
0.50	1.25	28.58	71.42	"	8.077
0.00	1.75	0.00	100	"	7.639
1.50	0.00	100	0.00	0.00959	8.589
1.20	0.30	80.0	20.0	"	7.885
0.75	0.75	50.0	50.0	"	7.107
0.30	1.20	20.0	80.0	"	6.612
0.00	1.50	0.00	100	"	6.336
1.25	0.00	100	0.00	0.00800	7.152
1.00	0.25	80.0	20.0	"	6.431
0.60	0.65	48.0	52.0	"	5.760
0.25	1.00	20.0	80.0	"	5.305
0.00	1.25	0.00	100	"	5.003
1.00	0.00	100	0.00	0.00641	5.642
0.75	0.25	75.0	25.0	"	4.972
0.50	0.50	50.0	50.0	"	4.452
0.25	0.75	25.0	75.0	"	4.042
0.00	1.00	0.00	100	"	3.714

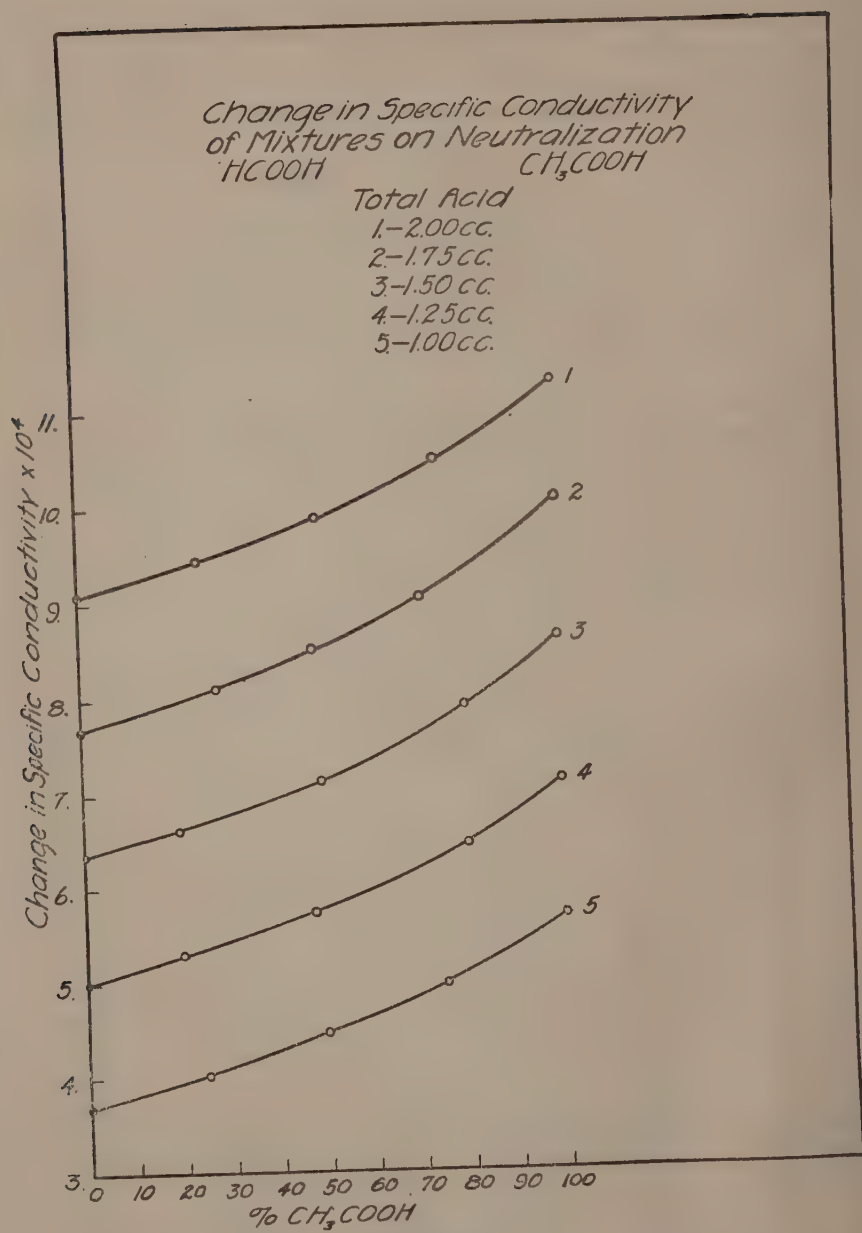


Fig. 5

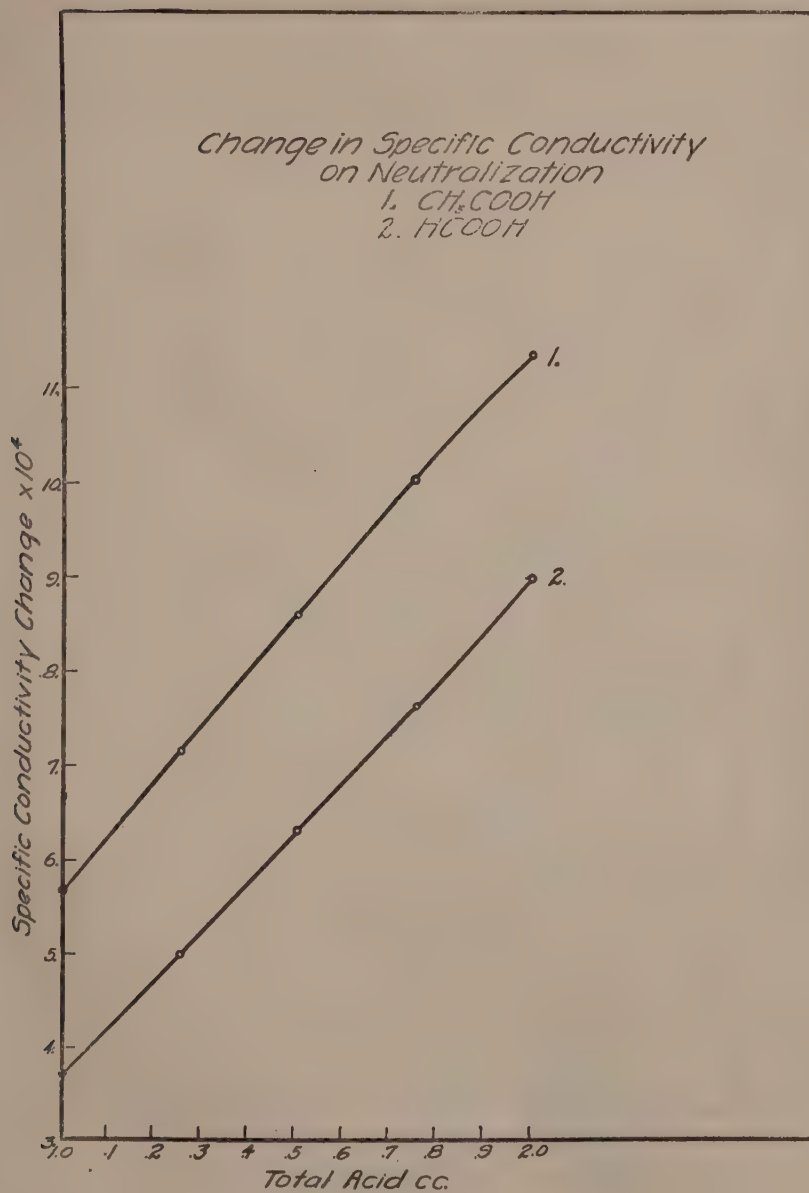
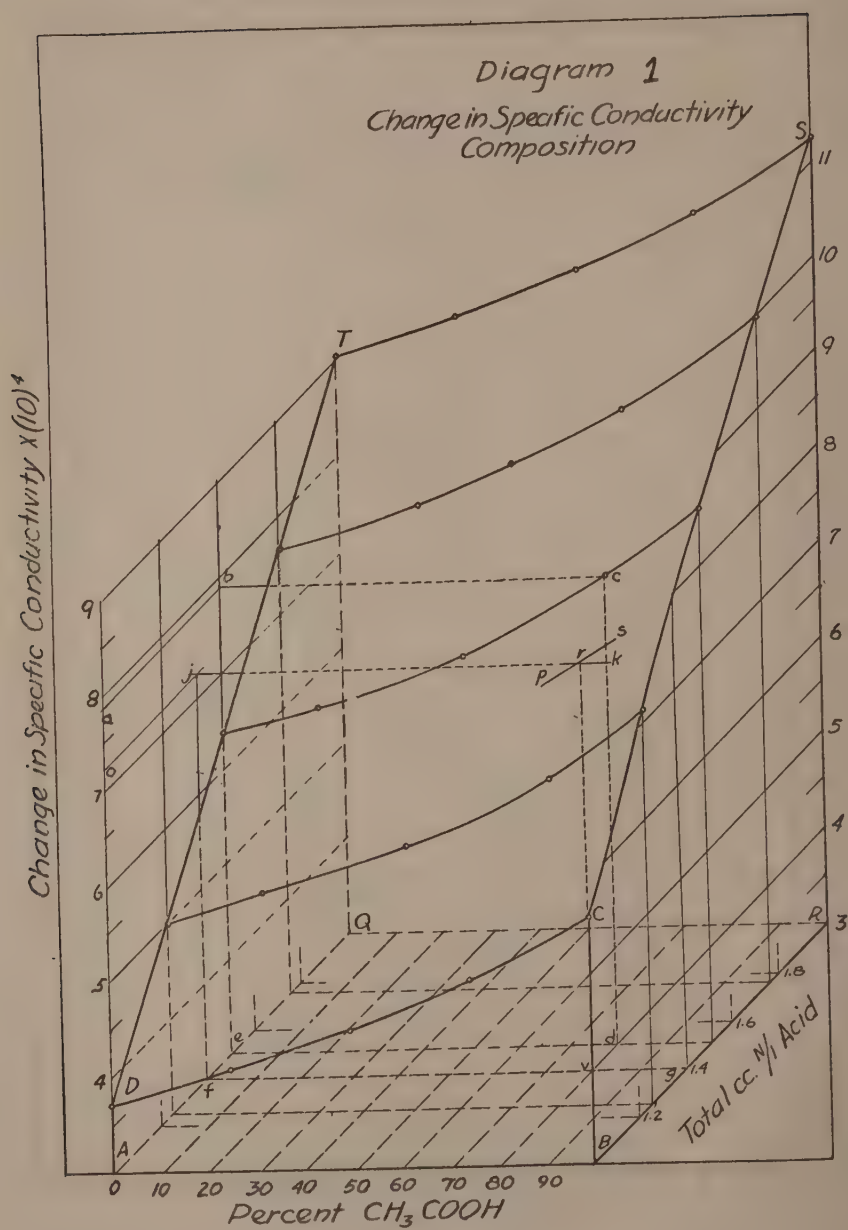


Fig. 6





plane represents the same ratio of acids, but different total acid composition, that is, the mol fraction is a constant for each plane, but the sum of the c.c. N acids varies from one to two. The profile plane on the left, AQTD, shows the total changes in specific conductivities of different concentrations of pure formic acid. The profile plane on the right, BRSC, refers to pure acetic acid. The profile planes between the above two represent different mixtures as indicated. For example, a plane parallel to the above two and midway between them would show the total changes in the specific conductivities of mixtures of the two acids in which the quantities of each are equal.

On the horizontal plane, ABRQ, the Z axis represents the total quantity of normal acid present, the X axis the ratio of the two acids.

The use of three component diagrams would seem to deserve wider use than it has received in cases where it is not possible to derive simple equations or where equations are derived with certain assumptions which may not be fully legitimate. The diagram is constructed from a number of points which are determined experimentally, each curve being determined by these points, so that no assumptions are made in its construction. The diagram proposed may be criticized on the grounds that it is not drawn to scale, that in the front planes ten centimeters represents only 1.00 cubic centimeter of total normal acid, while in the rear planes it represents 2.00 cubic centimeters. While it is true that the diagram does not give as good a picture of the actual conditions as one drawn to scale by making the X axis of the front plane half the length of the corresponding axis of the rear plane, such diagrams are more difficult to read than the one given. Furthermore, no assumption, and therefore no error, has been made in its construction. Each profile plane represents a certain mol fraction, each vertical plane a certain total acid composition and the horizontal plane has its values clearly marked so that each datum plotted has the significance indicated in Table 4.

## VI. THE USE OF THE CURVES AND DIAGRAM

The detailed procedure may best be illustrated by an example. It was found that 9.95 c.c. of a solution known to contain only formic acid and acetic acid required 1.20 c.c. of 1.159 N sodium hydroxide for neutralization with phenolphthalein as indicator, the acid being then 0.1399 N. There would be required 10.73 c.c. of the acid mixture to be equivalent to 1.50 c.c. N. This quantity was chosen because the curve for this strength is in the center of the range used and a slight error in dilution gives the minimum experimental error. The 10.73 c.c. of the acid solution were placed in the conductivity cell and 145.80 c.c. of dilution water making the total volume 156.5 c.c. on which the curve was based. The specific conductivity of this solution was  $2.527 \times 10^{-4}$ . It was found that 1.59 c.c. of N/1 ammonium hydroxide were required to just reach a constant value of specific conductivity, in this case  $10.412 \times 10^{-4}$ , whence the total change in specific conductivity was  $7.885 \times 10^{-4}$ . Dividing the value 1.59 c.c. by the factor 1.06 mentioned above showed the solution to contain the required 1.50 c.c. of N acid.

To locate the position of the solution on the diagram mark off on the horizontal edge of a card the distance, Aa, representing the value

$7.885 \times 10^{-4}$  found for the total change in specific conductivity. Slide the lower horizontal edge of the card along the 1.50 c.c. line, ed, until the length marked off on the edge of the card corresponds to the length, Ed, on the curve for this total concentration. The card intersects the plane A B R Q at the point d representing the composition of the solution which can be read on the diagram. In the example given, the mixture consisted of 80% acetic acid and 20% formic acid. The original solution was, therefore,  $0.80 \times 0.1399 = 0.1112$  N with respect to acetic acid and  $0.20 \times 0.1399 = 0.0279$  N with respect to formic acid.

The same conclusions as the above would be reached by the direct use of curve 17 in Figure 5. In fact, the curves in Fig. 5 can be used directly for solutions in which the exact concentrations are used for which the curves were derived. The use of the diagram is, however, not limited to the five exact concentrations plotted since on the Z axis, B R may be read the total changes in specific conductivity for intermediate concentrations. For example, let us assume that 10.00 c.c. of the solution discussed above were used instead of the 10.73 c.c. required to represent 1.5 c.c. of N. acid and the following data were obtained,

Initial specific conductivity  $= 2.401 \times 10^{-4}$

Final specific conductivity  $= 9.645 \times 10^{-4}$

Total change in specific conductivity  $= 7.244 \times 10^{-4}$

1.47 c.c. of 1N, ammonium hydroxide were required to bring the specific conductivity to a constant value.

$1.47/1.06 = 1.39$  as the number of c.c. of N acid present in the 156.5 c.c. The composition is read as previously along the line fj to jr, to rv again giving the compositions of 80% acetic acid and 20% formic acid. This was done on the assumption that each point on the 1.39 c.c. curve occupies a position  $14/25$  of the distance between the 1.25 c.c. curve and the 1.50 c.c. curve. While this assumption is not absolutely correct, deviations were found to be within the usual experimental error of the method.

## VII. SUMMARY

A method has been developed for the analysis of a mixture of formic acid and acetic acid in solution. The procedure is based on the fact that on the addition of the weak base, ammonium hydroxide, the specific conductivity at first increases and then reaches a constant value. The difference between the initial and final constant value of the specific conductivity is a function of the ratio of the concentrations of the two acids at a given total normality. A three way diagram has been constructed and its use explained in the analysis. The detailed method is accurately applicable to solutions or distillates known to contain only formic and acetic acids. The principles involved are being extended to other mixtures.



## LITERATURE CITED

- AUERBACH, F. UND PUDDLEMAN, W.  
1910. Massanalytische Bestimmung von Ameisensäure und ihren Salzen. *Arb. Kais. Gesundheitsamt* 30:178-94.
- AUERBACH, F. UND ZEGLIN, H.  
1922. Beiträge zur Kenntnis der Ameisensäure I, *Ztschr. Phys. Chem.* 103:161-77.
- BACON, R. F.  
1911. The detection and estimation of small quantities of ethyl and methyl alcohol and of formic acid. *Circ. U. S. Bur. Chem.* 74.
- BENEDICT, E. M. AND HARROP, G. A.  
1922. The estimation of formic acid in urine. *Jour. Biol. Chem.* 54:443-50.
- CUNY, L.  
1926. Dosage iodométrique de quelques acides organiques. *Jour. Pharm. Chim.* (8) 3:112-116.
- DONKER, H. L. J.  
1926. Bijdrage tot de Kennis der Boterzuur-Butyl-alcohol-en Acetongistungen. Thesis—Delft. (p. 42).
- DUCLAUX, M. E.  
1895. Sur le dosage des alcools et des acides volatils. *Ann. Inst. Pasteur* 9:265-80.
- DYER, D. C.  
1917. A new method of steam distillation for the determination of the volatile fatty acids, including a series of colorimetric qualitative reactions for their identification. *Jour. Biol. Chem.* 28:445-73.
- EASTMAN, E. D.  
1925. The theory of certain electrometric and conductometric titrations. *Jour. Am. Chem. Soc.* 47:332-7.
- FENTON, H. J. H. AND SISSON, H.  
1909. The action of metallic magnesium on certain aliphatic acids and the detection of formic acid. *Proc. Camb. Phil. Soc.* 14:385.
- FINCKE, K.  
1913. Beiträge zur Bestimmung der Ameisensäure in Nahrungsmitteln. *Ztschr. Unter Nahn. Genussm.* 25:386-90.
- FOUCHET, A.  
1912. Dosage de l'acide formique seul ou en mélange avec des homologues par le moyen du permanganate de potassium en milieu alcalin. *Ann. Chim. Anal.* 17:206-9.
- FRANZEN, H. UND EGGAR, F.  
1911. Zur quantitativen Bestimmung der Ameisensäure. *Jour. Prakt. Chem.* 83:325-5.
- FRANZEN, H. UND GRIEVE, G.  
1909. Über die quantitativen Bestimmung der Ameisensäure. *Jour. Prakt. Chem.* 80:368-89.
- GROSSMAN, H. UND AUFRICHT, A.  
1906. Titrimetrische Bestimmung der Formaldehyd und Ameisensäure mit Kaliumpermanganat in saurer Lösung. *Ber. Deut. Chem. Gesell.* 39:2455-8.
- HEERMANN, P.  
Über die Trennung von Ameisen und Essigsäure. *Chem. Ztg.* 39:157.
- HEUSER, R.  
1915. Die Bestimmung von Ameisen und Essigsäure in sehr verdünnten Lösungen. *Chem. Ztg.* 39:57-59.

HOLMBERG, B. UND LINDBERG, S.

1923. Titrimetrische Bestimmung der Ameisensäure. Ber. Deut. Chem. Gesell. 56B:2048-52.

HOTTENROTH, V.

1914. Bestimmungsmethode für Ameisensäure. Chem. Ztg. 38:598.

JOSEPH, A. P.

1910. The estimation of formic acid. Jour. Soc. Chem. Ind. 29:1189-90.

KLEIN, J.

1906. Die Bestimmung der Ameisensäure mit Kaliumpermanganat. Ber. Deut. Chem. Gesell. 39:2640-1.

KOHLRAUSCH, F. UND HOLBERN, L.

1898. Das Leitvermögen der Electrolyte. B. G. Teubner, Leipzig. p. 25.

KOLTHOFF, I. M.

1923. Konduktometrische Titrationsen. Theodor Steinkopff, Dresden und Leipzig.

KRAUS, CHAS. A.

1922. The properties of electrically conducting solutions. Chem. Cat. Co., New York, p. 43.

LAUFFMANN, R.

1915. Bestimmung von Ameisensäure neben Essigsäure. Chem. Ztg. 39:575.

LEEDS AND NORTHRUP

1925. The Vreeland oscillator. Bull. Leeds and Northrup Co. 894.

LIEBIG, J.

1849. Ueber die Trennung einiger Säuren der Reihe  $(\text{CH})_n\text{O}_4$ . Liebigs Ann. Chem. 71:355-7.

MACNAIR, D. C.

1887. Note on the separation of formic and acetic acids. Chem. News 55:229.

NICLAUX, M.

1897. Dosage de petite quantites d'alcohol methylique, d'aldehyde formic d'acid formique. Bull. Soc. Chim. de Paris 17:839-40.

OBERHAUSER, F. AND HENSINGER, W.

1927. Ueber die Quantitativen Bestimmung der Ameisensäure. Ztschr. Anorg. u. Allg. Chem. 160:366-72.

OST, H. UND KLEIN, F.

1908. Ameisensäure in Eisessig. Chem. Ztg. 32:815-6.

REILLY, J. AND HICKENBOTTOM, W. J.

1919. The determination of volatile fatty acids by an improved distillation method. Sci. Proc. Roy. Dublin Soc. 15:513-38.

REILLY, J., RAE, W. N., AND WHEELER, T. S.

1925. Physico-Chemical methods. Van Nostrand Co., New York, p. 577.

RITHMOND, H. T.

1895. Duclaux's method for the estimation of the volatile fatty acids, the laws governing volatility deduced therefrom and their application to analysis, especially to that of butter. Analyst 20:193-8, 217-9.

TAYLOR, W. A. AND ACREE, S. F.

1916. Studies in the measurement of the electrical conductivity of solutions at different frequencies. Jour. Am. Chem. Soc. 38:2396-2430.

TSIROPINAS, F.

1917. A volumetric method for the determination of formic acid or formates in the presence of hydroxides, carbonates, oxalates and acetates. *Jour. Ind. Eng. Chem.* 9:1110-1.

UPSON, F. W., PLUM, H. H. AND SCHOTT, J. H.

1917. On the Duclaux method for the estimation of volatile fatty acids. *Jour. Am. Chem. Soc.* 39:731-46.

UTRIK-LJUBOWZOFF, L.

1923. A new titrimetric method for estimating formic acid. *Biochem. Jour.* 138:205-8.

WEGENER, M.

1903. Gasometrische Bestimmung der Ameisensäure und ihren Salzen. *Ztschr. Anal. Chem.* 43:427-31.

WHITTIER, E. O.

1923. Determination of formic acid. *Jour. Am. Chem. Soc.* 45:1087.

WOHACK, F. Z.

1921. Mikro-analytische Verfahren in der Nahrungsmitteluntersuchung. *Ztschr. Unter Genussm.* 42:290-8.





# SOME CORRELATIONS OF CONSTITUTION WITH SWEET TASTE IN THE FURAN SERIES<sup>1</sup>

BY HENRY GILMAN AND AMIOT P. HEWLETT

*From the Chemical Laboratory of Iowa State College*

Accepted for publication July 11, 1929.

There is a need for synthetic sweetening agents. Perhaps the best known compound of this type is saccharin. Others like dulcin are also used. The sweetest compound so far described is the *anti*-oxime of perilla aldehyde<sup>2</sup>. This compound is 2,000 times sweeter than cane sugar. Its structure is not definitely known. The most interesting fact concerning this compound, from a stereo-chemical viewpoint, is that its *syn* form is tasteless. In connection with the present studies it is also interesting to note that although nitriles, in general<sup>3</sup>, have a sweeter taste than the corresponding oximes, perilla nitrile is only 200 times sweeter than cane sugar.

Essentially no study has been made of taste and constitution in the furan series. Furonitrile has been listed<sup>4</sup> as having a sweetish taste and furylalanine as being very sweet. Kanao<sup>4</sup> synthesized a number of nitro alcohols of the furan series by condensation of furfural with nitro-paraffins and reported that they had a sweet taste.

Hill and Sylvester<sup>5</sup> attempted, without success, the preparation of the furan analogue of saccharin: namely, furoic sulfinide. A part of the work described at this time is patterned after dulcin<sup>6</sup>. However, the compounds which gave the sweetest taste are furonitrile and furylacrylonitrile. The former is 100 times sweeter than sugar and the latter 200 times sweeter than sugar. Attention is directed in the Experimental Part to a miscellany of correlations between sweet taste and constitution of other furan types. Also, a check or reference method is described for the determination of the relative sweetness of compounds.

---

<sup>1</sup>This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge assistance from the Industrial Science Research Fund for the defrayal of expenses incurred in this investigation.

<sup>2</sup>Furukawa, *J. Tokyo Chem. Soc.*, **41**, 726 (1920). *C. A.* **15**, 1145 (1921).

<sup>3</sup>General references to taste and constitution may be found in the excellent books by G. Cohn entitled "Die organischen Geschmackstoff" (Berlin, 1914) and "Geschmack und Konstitution" (Berlin, 1915). See, also, the interesting chapter on taste and constitution in Fraenkel, "Die Arzneimittel-Synthese". Some recent articles are by Oertley and Meyers, *J. Am. Chem. Soc.*, **41**, 855 (1919); Magidson and Gorbachov, *Ber.*, **56B**, 1810 (1923); and Dox and Jones, *J. Am. Chem. Soc.*, **50**, 2033 (1928).

<sup>4</sup>Kanao, *J. Pharm. Soc. Japan No.* **550**, 1019 (1927). *C. A.*, **22**, 1588 (1928).

<sup>5</sup>Hill and Sylvester, *Am. Chem. J.* **32**, 185 (1907).

<sup>6</sup>Leading references to the effect of alteration of the dulcin molecule on taste may be traced back from the recent article by Lorang, *Rec. trav. chim.*, **47**, 179 (1928). See also, Ref. 3.

## EXPERIMENTAL PART

1. *Furfuryl Urea*

**FURFURALDOXIME**,  $C_4H_3OCH=NOH$ . The method of Goldschmidt<sup>7</sup> was used. A solution of 31.4 g. (0.78 mole) of sodium hydroxide in 100 c.c. of water was added to 11 g. (0.16 mole) of hydroxylamine hydrochloride in 50 c.c. of water. Ten g. (0.104 mole) of furfural was added to this solution, and after standing for 12 hours the mixture was almost neutralized with hydrochloric acid and then saturated with carbon dioxide. The ether layer obtained by extraction of this mixture was separated; dried over anhydrous sodium carbonate; filtered; and then the ether was removed by distillation, leaving an oily residue which crystallized on standing. It melted at 50-60°, and was evidently a mixture of the two forms of the oxime. A small quantity of the *anti*-oxime, melting at 73°, was secured by recrystallizing several times from petroleum ether.

The larger portion of the mixture was converted entirely to the *syn*-form, which melts at 89°. This was done by dissolving the oxime in dry ether and saturating with hydrogen chloride gas, which precipitated the oxime as the hydrochloride. This was filtered, washed with ether, placed in a flask and covered with ether. To this mixture a saturated solution of sodium carbonate was added cautiously to an alkaline reaction. The ether layer was removed, dried, and then most of the ether was distilled off, leaving a residue which when crystallized from petroleum ether melted at 89°. The yield was 8.6 g. or 75%.

**FURFURYL AMINE**,  $C_4H_2OCH_2NH_2$ . To 10 g. (0.105 mole) of the oxime in 200 c.c. of absolute alcohol warmed to 50° under reflux, 40 g. (1.7 atom) of sodium was added in small pieces as rapidly as reaction would permit. The mixture was then allowed to cool, and the reaction product was dissolved in water. After making distinctly acid with 10% sulfuric acid, the alcohol was removed by distillation under reduced pressure. The residue was made alkaline by adding solid sodium hydroxide, and the ether extract of the base was dried over calcium chloride and distilled. The furfuryl amine was obtained as a stable, colorless liquid, of an extremely offensive odor. It distilled at 145° at atmospheric pressure and at 70°/7 mm. The yield was 3.5 g. or 40%. It is readily converted to its hydrochloride by precipitation from an ether solution by dry hydrogen chloride.

**FURFURYL UREA**,  $C_4H_3OCH_2NHCONH_2$ . An unsuccessful attempt was made to prepare the pure urea in accordance with the method of Berlinerblau<sup>8</sup> for the synthesis of dulcin. A saturated solution of potassium cyanate was added to an equimolecular saturated solution of furfurylamine hydrochloride in water. No precipitate formed even after standing for some time. The distinctly alkaline solution was extracted with ether, but on removal of the ether by evaporation only furfurylamine was recovered.

On removal of the water from the solution by evaporation under reduced pressure, a white residue which rapidly became gummy remained. This was extracted with alcohol, and on evaporation of the alcohol white crystals were formed. These melted at 105°, but rapidly changed to a

<sup>7</sup>Goldschmidt, *Ber.* 25, 2582 (1892).



gummy mass on standing. The compound, which may have been the desired furfuryl urea, did not have a sweet taste.

## 2. Phenyl-Furylmethyl Urea

**FURYL PHENYL KETONE**,  $C_4H_3OCC_6H_5$ . This ketone was syn-



thesized by the method of Marquis<sup>9</sup>. To 44 g. (0.42 mole) of anhydrous aluminum chloride, covered with benzene and contained in a round-bottomed flask, was slowly added 40 g. (0.31 mole) of furoyl chloride dissolved in an equal volume of benzene. The reaction started when warmed to 50°, and when evolution of hydrogen chloride ceased the mixture was poured on to 500 g. of fine ice in order to decompose the complex. The benzene layer was separated, washed with sodium hydroxide, dried over calcium chloride, and distilled. The yield of furyl phenyl ketone boiling at 175°/6 mm. was 44 g. or 85%.

**FUROYL CHLORIDE**,  $C_4H_3OCCl$ . The furoyl chloride was prepared from 1.5 moles of thionyl chloride in 500 c.c. of dry benzene and 96 g. (one mole) of furoic acid. After refluxing for 12 hours, the mixture was filtered and distilled, the acid chloride being obtained in a 97.5 g. or 75% yield as a colorless liquid boiling at 176°. It is quite stable when pure, and is very disagreeable to work with because of its pronounced lachrymatory properties, which will be reported later with related studies.

**OXIME of FURYL PHENYL KETONE**. This was also prepared by the method of Marquis<sup>9</sup> from 10 g. of ketone in 200 c.c. of alcohol and 6 g. of hydroxylamine hydrochloride in 50 c.c. of water. After making the mixture alkaline by the addition of 6 g. of sodium hydroxide in 10 c.c. of water, it was allowed to stand for 24 hours; diluted to a total volume of 2 liters; and acidified with acetic acid to precipitate the oxime. When filtered, dried and crystallized from ether it melted at 132-133°, and the yield was 9.7 g. or 90%. The oxime is without sweet taste.

**FURYL-PHENYL-METHYLAMINE**,  $(C_4H_3O)(C_6H_5)CHNH_2$ . To 10 g. (0.054 mole) of the oxime in 200 c.c. of absolute alcohol heated to reflux, was added 40 g. (1.7 atoms) of sodium in small pieces as rapidly as the reaction would permit. The product obtained on cooling was dissolved in water and made acid with 10% sulfuric acid. The residue obtained, after removal of alcohol by distillation under reduced pressure, was made alkaline with solid sodium hydroxide. The ether extract of this was dried over calcium chloride and distilled to yield 5.5 g. or 60% of the amine boiling at 144-145°/17 mm. Marquis<sup>9</sup> gives the boiling point as 167-168°/43-44 mm. The amine was identified as the acetyl derivative which melted at 127°. The known amine hydrochloride was prepared in the usual manner by precipitation from an ether solution with hydrogen chloride.

**FURYL-PHENYL-METHYLUREA**,  $(C_4H_3O)(C_6H_5)CHNHCONH_2$ . The urea was prepared by Berlinerblau's<sup>8</sup> method by adding a saturated solution of potassium cyanate to the amine hydrochloride. The white precipitate which formed immediately was filtered, dried, and crystallized

<sup>8</sup>Berlinerblau, *J. prakt. chem.*, (2) 30, 103 (1884).

<sup>9</sup>Marquis, *Ann. chim. phys.*, (8), 4, 276 (1905).

from alcohol. It melted at  $80^{\circ}$ , had a bitter, acrid taste and was not at all sweet. It decomposes on standing, evolving ammonia and leaving a brownish insoluble substance. Unfortunately, no analysis of it was made because of its apparent instability.

### 3. *Furyl-p-Phenetyl-Methylurea*

**FURYL-*p*-PHENETYL KETONE.** This ketone was prepared in accordance with the method previously given in this paper for the preparation of furyl phenyl ketone, from furoyl chloride, phenetole and aluminum chloride in carbon disulfide. It distilled at  $220^{\circ}/25$  mm. and melted at  $70^{\circ}$ . When oxidized with neutral potassium permanganate it gave *p*-ethoxybenzoic acid, melting at  $195^{\circ}$ .

*Analysis.* Calcd. for  $C_{13}H_{12}O_3$ : C, 72.22, H, 5.55. Found: C, 72.02; H, 5.62%.

**OXIME of FURYL *p*-PHENETYL KETONE** ( $C_4H_3O$ ) ( $p$ - $C_2H_5OC_6H_4$ ) $C=NOH$ . This oxime was synthesized in the customary manner from the ketone and hydroxylamine hydrochloride. It melted at  $145^{\circ}$  and was obtained in an 80% yield. It was without sweet taste.

*Analysis:* Calcd. for  $C_{13}H_{13}O_3N$ : C, 67.53; H, 5.62. Found: C, 67.99%; H, 5.78%.

**FURYL-*p*-PHENETYL-METHYLAMINE**, ( $C_4H_3O$ ) ( $p$ - $C_2H_5OC_6H_4$ ) $CHNH_2$ . The method used for the attempted preparation of this pure amine was the reduction of the oxime by sodium and alcohol. The yield of product was low, 0.95 g. or about 10% of the amine being obtained from 10 g. of the oxime. No attempt was made to distill the unstable amine. Instead, its hydrochloride was formed by adding hydrogen chloride to an ether solution of the supposed amine. The hydrochloride was also unstable, decomposition setting in immediately on air exposure resulting subsequently in the formation of a rubber-like mass which finally became very brittle.

**FURYL-*p*-PHENETYL-METHYLUREA.**—Because of the instability of the amine and its hydrochloride, the latter was used immediately on its removal from ether. On addition of the probable amine hydrochloride to a saturated solution of the calculated quantity of potassium cyanate, a white precipitate formed. This compound, which may have been the desired urea, was practically tasteless, and had no sweet taste. It underwent decomposition on standing, with the evolution of ammonia. Partial decomposition took place on crystallization from alcohol. No analysis was made.

### 4. *Furyl-p-Bromophenyl-Methylurea*

**FURYL *p*-BROMOPHENYL KETONE**, ( $C_4H_3O$ ) ( $p$ - $BrC_6H_4$ ) $C=O$ . This ketone was prepared from furoyl chloride, bromobenzene and aluminum chloride in carbon disulfide. The yield of ketone distilling at  $175$ – $177^{\circ}/7$  mm. was 49%. When oxidized it gave *p*-bromobenzoic acid, melting at  $250^{\circ}$ .

*Analysis.* Calcd. for  $C_{11}H_7O_2Br$ : Br, 32.22. Found: Br, 32.12 and 32.08%.

**OXIME OF FURYL *p*-BROMOPHENYL KETONE**,  $(\text{C}_4\text{H}_3\text{O})(p\text{-BrC}_6\text{H}_4)\text{C}=\text{NOH}$ .—The yield of oxime from 10 g. of the ketone was 10 g or 94%. It melted at  $122^\circ$ , and was without sweet taste.

*Analysis.* Calcd. for  $\text{C}_{11}\text{H}_5\text{O}_2\text{NBr}$ : Br, 30.42. Found: Br, 30.30%.

**FURYL-*p*-BROMOPHENYL-METHYLAMINE.** The oxime when reduced in the previously described manner with sodium and alcohol, gave a poor yield of a heavy oil which was probably the amine. Like some other amines reported in this study it darkened immediately and decomposed to a black, apparently intractable mass. It was immediately converted to the hydrochloride in the attempted synthesis of the related urea, even though the hydrochloride underwent apparent rapid decomposition with the liberation of hydrochloric acid.

**FURYL-*p*-BROMOPHENYL-METHYLUREA.**—The usual reaction between the amine-hydrochloride and potassium cyanate gave a colorless solid, which soon decomposed with the liberation of ammonia. The compound was tasteless, and a Beilstein test showed halogen to be present. No analysis was made.

#### 5. *Omega*-Furyl-Allyl Urea, $\text{C}_4\text{H}_3\text{OCH}=\text{CHCH}_2\text{NHCONH}_2$

**OXIME of FURYLACROLEIN**,  $\text{C}_4\text{H}_3\text{OCH}=\text{CHCH}=\text{NOH}$ . The oxime, melting at  $124^\circ$ , was obtained in a 75% yield (10.3g.) from 12.2 g. of the ketone.<sup>10</sup> This oxime has a very slight sweet taste, but less than that of the oximes of furfural.

**FURYL-ALLYL AMINE**,  $\text{C}_4\text{H}_3\text{OCH}=\text{CHCH}_2\text{NH}_2$ . This unstable amine was obtained in a poor yield by the sodium alcohol reduction of the corresponding oxime. It was converted immediately to the hydrochloride which was used in the attempted preparation of the urea.

Again, this particular urea was found to be unstable. It is slightly soluble and without a sweet taste.

#### 6. *Furylacrylonitrile*

**FURYLACRYLOYL CHLORIDE**,  $\text{C}_4\text{H}_3\text{OCH}=\text{CHCOCl}$ . This acid chloride was prepared by the method used for furoyl chloride. A solution of 1.5 moles of thionyl chloride in 500 cc. of benzene was added to 138 g. (one mole) of solid furylacrylic acid. After refluxing for 3 hours, the benzene was distilled off, and the acid chloride distilled in a vacuum (water-pump) at  $145^\circ/30\text{mm}$ . The yield was 141 g. or 90%. It apparently can be kept indefinitely in an ice-box, and undergoes but slight decomposition when sealed up in a pure condition.

**FURYLACRYLAMIDE**,  $\text{C}_4\text{H}_3\text{OCH}=\text{CHCONH}_2$ . In a 0.1 mole run a 90% yield of amide,<sup>11</sup> melting at  $168^\circ$ , was obtained by saturating an ether solution of the acid chloride with ammonia gas.

**FURYLACRYLONITRILE**,  $\text{C}_4\text{H}_3\text{OCH}=\text{CHCN}$ . The synthesis was carried out in accordance with the preparation of furonitrile described later in this study. However, on warming the mixture of amide and phosphorus pentachloride, a vigorous decomposition took place. Instead of a distilla-

<sup>10</sup>This oxime was prepared previously by König, *Ber.*, 58B, 2559 (1925).

<sup>11</sup>Rinkes, *Rec. trav. chim.*, 39, 201 (1921).



tion, the tarry mass was extracted with ether to obtain the nitrile which was identified by hydrolysis to furylacrylic acid, the identity of which was confirmed by a mixed melting point determination with an authentic specimen.

*Analysis.* Calcd. for  $C_7H_5ON$ : N, 11.79. Found N, 11.89 and 11.57%.

Some orienting experiments were made to improve the yield. These involved the use of phosphorus pentoxide, zinc chloride, and acetic anhydride as dehydrating agents. Also, a mixture of zinc furylacrylate was heated with lead thiocyanate, but with indifferent success.

Finally, it was found that a 30% yield could be obtained from an intimate mixture (by shaking) of 2 moles of finely powdered phosphorus pentachloride and 1 mole of finely divided amide. Reaction sets in at room temperature and is accompanied by a charring of the mixture and an elevation in temperature. At this point, the flask should be placed in ice to guard against the temperature rise. After about 15 minutes, the mass is extracted with ether, and the ether solution is thoroughly washed with water, dried and distilled. The nitrile boils at  $100^\circ/5$  mm. and melts at  $32^\circ$ . After standing for a few days it darkens somewhat, and the melting point is depressed. However, no deep-seated decomposition appears to take place and the compound keeps fairly well. It remains colorless when kept in an ice-box. It was found that a saturated solution contains 0.31% of the nitrile at  $30^\circ$ .

#### 7. Furonitrile, $C_4H_3OCN$

*FURAMIDE*,  $C_4H_3OCONH_2$ . The amide was prepared<sup>12</sup> by saturating an ether solution of the acid chloride with ammonia. The solid which is filtered consists mainly of the amide, but contains appreciable quantities of ammonium chloride. The amide may be purified by crystallization from alcohol or benzene. The ether solution contains a small amount of amide which may be recovered by evaporation of the ether. The amide which melted at  $142^\circ$  was obtained in a 95% yield (10.5 g. from 13 g. of the acid chloride).

The furonitrile was prepared from the amide by the method of Wallach<sup>13</sup> in a 70% yield from a 0.1 mole run. A saturated solution at  $30^\circ$  contains 31% of the nitrile.

#### METHOD OF TESTING SWEETNESS

It has long been known that the sense perception of taste in individuals varies. We are aware of no standard method for tasting.<sup>14</sup> When we say, for example, that saccharin is 675 times as sweet as cane sugar we mean that this is an average result based on many taste tests. In our tests involving the relative sweetness of compounds we used a very simple check

<sup>12</sup>Ciamician and Dennstedt, *Ber.*, 14, 1059 (1881).

<sup>13</sup>Wallach, *Ann.*, 214, 288 (1882).

<sup>14</sup>The reader can get leading references in the standard works given in Footnote 3 of this paper. Perhaps one of the most accurate and satisfactory methods is described by Pauli, *Biochem. Ztg.*, 125, 97 (1921); *C. A.*, 16, 975 (1922). Another more recent method is by Willaman, *Z. Ver deut. Zuckerind.* (1927) p. 365-7 (*C. A.*, 22, 1784 (1928)).

method which, so far as we know, is novel. In order to make certain that there were no taste abnormalities in those who made the tests, and also in order to assure ourselves that we were making our comparative tests on a sound basis, we not only determined the relative sweetness of our compounds by comparing them with *several* standard sweet tasting compounds, but also checked the known and approximate relative sweetness of the standards—one against the others.

That is, our compounds were compared not only with sugar but also with saccharin and dulcin. Furthermore, the saccharin and dulcin were compared not only with sugar but also with each other. In this manner we obtained a series of cross checks which, with the known standards, agreed with the average results reported in the literature and so confirmed the correctness of our procedure.

By code numbers and by occasionally rearranging the positions of the several samples, the experimenters who did the testing were quite ignorant of the solutions tested. A 2% sugar solution was used as a standard, and the nitrile solutions varied in concentration from 1:3,000 to 1:50,000.

On an actual gram weight basis, it was found that furylacrylonitrile was 200 times as sweet as sugar, 0.75 as sweet as dulcin, 0.33 as sweet as saccharin and twice as sweet as furonitrile. The furonitrile was found to be 0.5 as sweet as furylacrylonitrile, 0.40 as sweet as dulcin and 0.15 as sweet as saccharin. Dulcin was found to be 265 times as sweet as sugar and 0.39 as sweet as saccharin. Saccharin was found to be 675 times as sweet as sugar under our particular conditions. Each of the two furfuraldoximes was found to be 2.5 times as sweet as sugar, and this value was obtained by comparison with sugar only.

None of these compounds has been tested for its physiological action on animals. Such tests will wait on the probability of securing sweeter tasting furan types.

The authors wish to thank the Miner Laboratories of Chicago for liberal supplies of furfural, and J. Dickey and G. F. Wright for their assistance in evaluating the relative sweetness of the compounds prepared in this study.

### SUMMARY

A study has been made of the relative sweetness of several furan compounds. It has been found that furylacrylonitrile, furonitrile and the furfuraldoximes are respectively 200, 100 and 2.5 times as sweet as sugar. A simple method is suggested for checking the reliability of technique in comparing the sweet taste of new compounds against standards like sugar, saccharin and dulcin.

Ames, Iowa.





# THE PREPARATION OF FURFURAL DIACETATE<sup>1</sup>

BY HENRY GILMAN AND GEORGE F. WRIGHT

*From the Department of Chemistry, Iowa State College.*

Accepted for publication July 11, 1929.

There is a need for furfural diacetate. In some of our studies we have found this compound extremely useful in direct substitution reactions (bromination, nitration, etc.) leading to the preparation of new and interesting, and useful types<sup>2</sup>.

Furfural diacetate was prepared previously by others. Law<sup>3</sup>, who first synthesized it, used catalytic acetylation methods, but gave no mention of yields. Blanksma<sup>4</sup> also recorded no yields. Knoevenagel<sup>5</sup>, in a study of the relative catalytic action of several salts, prepared it in very small quantities (3 to 4 grams) in about 70% yields. The most recent method is that of Scheibler, Sotscheek and Friese<sup>6</sup>. After reviewing the earlier work of others, they elected to prepare the diacetate by refluxing furfural with an excess of acetic anhydride and acetic acid. The 68% yield obtained by them (in a 0.78 mole run) is based on furfural. However, when based on acetic anhydride their yield is only 20%.

In our work we wished to prepare the diacetate in reasonably large quantities and at a low cost. Our 4 mole runs (the largest reported) gave uniform yields in several preparations, and can undoubtedly be extended to even larger sized runs either with no impairment of yield or in improved yields. Furthermore, we saw no object in using a large excess of acetic anhydride. Other things being equal, sound economies demands that that chemical be used in excess which is least expensive. Furfural is the lowest priced aldehyde, and one of the lowest priced organic chemicals, and certainly distinctly lower in price than acetic anhydride. Accordingly, we used practically equivalent quantities of furfural and of acetic anhydride. By the application of Knoevenagel's<sup>5</sup> extremely small-scale catalytic methods we have consistently prepared the diacetate in 4 mole runs with yields of about 43%. This method of preparation is not only less expensive than others, but also more convenient.

## EXPERIMENTAL PART

Three hundred and eighty-four grams (4.0 moles) of freshly distilled furfural, chilled to 0°, were added to 428 g. (4.2 moles) of acetic anhydride

<sup>1</sup>This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge help from the Industrial Science Research Fund for the defrayal of expenses incurred in this investigation.

<sup>2</sup>A preliminary account of some of these reactions was presented at the Iowa Academy of Science Meeting held at Fairfield, Iowa, on April 27, 1929.

<sup>3</sup>Law, *Chem. Ztg.*, 32, 365 (1908).

<sup>4</sup>Blanksma, *Chem. Weekblad*, 6, 717 (1909).

<sup>5</sup>Knoevenagel, *Ann.*, 402, 119 (1914).

<sup>6</sup>Scheibler, Sotscheck and Friese, *Ber.* 57, 1443 (1924).

contained in a three-necked two-liter flask and chilled to  $-5^{\circ}$ . One gram of stannous chloride dihydrate,  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  dissolved in the acetic anhydride, was used as a catalyst. The addition of furfural was effected as rapidly as possible to the well-stirred solution, care being exercised that the temperature did not rise above  $-3^{\circ}$ .

After standing for 24 hours the reaction mixture was poured into 2 liters of water and a sodium carbonate solution and ice were then added until coagulation of the oil was complete. After a few minutes the mixture was filtered by suction. After drying the crystalline product over night (in a vacuum desiccator over sulfuric acid) it was distilled under reduced pressure. The fraction distilling at  $138-144^{\circ}/20$  mm. weighed 41 g. This is 43.1% of the theoretical yield based on acetic anhydride. The compound as distilled *in vacuo*, melted at  $52-53^{\circ}$ .

The authors are grateful to the Miner Laboratories of Chicago for liberal supplies of furfural.

### SUMMARY

Improved directions are given for the convenient preparation of furfural diacetate in reasonably large quantities.

# ANTISEPTIC HYPOCHLORITE BY ELECTROLYSIS

BY R. L. VAN PEURSEM, B. K. POSPISHUL AND W. D. HARRIS\*

*From the Department of Chemical Engineering, Iowa State College*

Accepted for publication, August 1, 1929.

## INTRODUCTION

That sodium hypochlorite has antiseptic properties has been known for a long time—Eau de Javelle and Eau de Labarraque having been used for centuries. There has, however, been some controversy among medical men as to its practical efficiency as a germicide under ordinary first aid and hospital conditions. It was not until after the studies of Carrel and Dakin that sodium hypochlorite became popular. Their studies showed that the concentration of sodium hypochlorite must be exactly between 0.45 and 0.50 per cent. Below 0.45 per cent the solution is not sufficiently active to be efficient as an antiseptic and above 0.50 per cent it becomes irritating. The solution must also be free from causticity. In the first Dakin solution the causticity was eliminated by the addition of boric acid. When this was later found to be irritating to wounds it was replaced by sodium bicarbonate.

Sodium hypochlorite solution was formerly prepared by the reaction of sodium carbonate on a solution of bleaching powder. The bleach must be tested for available chlorine, the sodium carbonate must be pure, and the amounts of the materials must be balanced according to the available chlorine in the bleach. After the reaction is complete the solution must be filtered to take out the precipitate of calcium carbonate. At present the sodium hypochlorite solution is prepared by passing chlorine gas through a solution of sodium carbonate. With this method also, the amounts of chemicals must be accurately controlled. With either process the preparation must be made by an experienced man.

The sodium hypochlorite solution upon standing loses its antiseptic value even if kept carefully stoppered in a dark bottle. In order to secure the best results the solution should be used at the time of preparation, when its strength is the greatest. Efforts to stabilize the solution have not been successful. The chlor-organic compounds are not now considered as efficient.

It is the general belief that gaseous chlorine has the same antiseptic effect as the hypochlorous acid radical from Dakin's solution. There is, however, reason to believe that Dakin's solution has different properties than ordinary chlorine. The chlorine from Dakin's solution is available in a nascent state, which makes it more efficient.

---

\*A summary of research carried out in the Chemical Engineering Laboratories. Prepared for publication by Lionel K. Arnold, Engineering Experiment Station, Iowa State College.



Studies have been made of a method and apparatus to produce sodium hypochlorite solution of the proper concentration and pH value for anti-septic uses. The object of these studies was to produce an apparatus which would provide the surgeon with freshly prepared Dakin's solution of proper concentration and pH at a turn of a switch. It seemed evident that these conditions could be best obtained by some type of electrolytic cell, although electrolytic cells had not been used previously for this purpose. Hypochlorite for bleaching and other commercial purposes is made in electrolytic cells. It has been known since the patent of Charles Watt (English Patent No. 13755) that the following factors are necessary for a successful cell: (a) insoluble electrodes must be used in order to maintain an efficient cell; (b) no diaphragm is necessary; (c) a low temperature is necessary in order to prevent the formation of sodium chlorate; and (d) there should be a rapid circulation of electrolyte from the cathode to the anode. The commercial cells use carbon electrodes, platinum electrodes, platinum iridium electrodes, and magnesium electrodes.

### EXPERIMENTAL

One of the first electrolytic cells used had electrodes of platinum foil. The cathode was in the shape of a cylinder one inch in diameter and two inches high. The anode was  $\frac{5}{8}$  of an inch wide and about 4 inches long. It was placed within the cathode and extended almost the entire length of the main body of the cell. All connections within the cell were made of platinum. Current to operate the cell was supplied by a storage battery, which was kept charged with a battery charger. The amount of current was controlled by variable resistance and determined by an ammeter. A saturated salt solution was dropped from a dropping funnel into the cell. The salt solution used was prepared from chemically pure sodium chloride. Excess barium was removed with sodium carbonate and the solution made neutral to phenolphthalein with hydrochloric acid. The action of the cell is as follows: As soon as the salt solution enters the cell, electrolysis takes place. Chlorine gas is evolved at the anode, hydrogen at the cathode. The reactions are



The first run, using a current of 0.4 amperes, produced a solution of 0.179 per cent sodium hypochlorite. Since this is too weak for use as an antiseptic, several runs were made in an effort to increase the concentration. Cooling the cell with ice increased the concentration to 0.349, which was still too low. The addition of one per cent of the weight of sodium chloride in sodium chromate gave a concentration of 0.558 per cent, or more than necessary. By increasing the rate of feed and the current use, the concentration was increased. Since the solution prepared was alkaline, boric acid was added to the salt solution before electrolysis to decrease the alkalinity. This had very little effect.

A series of runs over periods of 4 hours each was made to determine the variation in concentration with length of run. During the second hour the concentration increased. During the last two hours it decreased, probably due to the rise in temperature of the cell. A similar series of runs

with electrodes reversed gave practically identical results. A summary of these various results is given in Table 1.

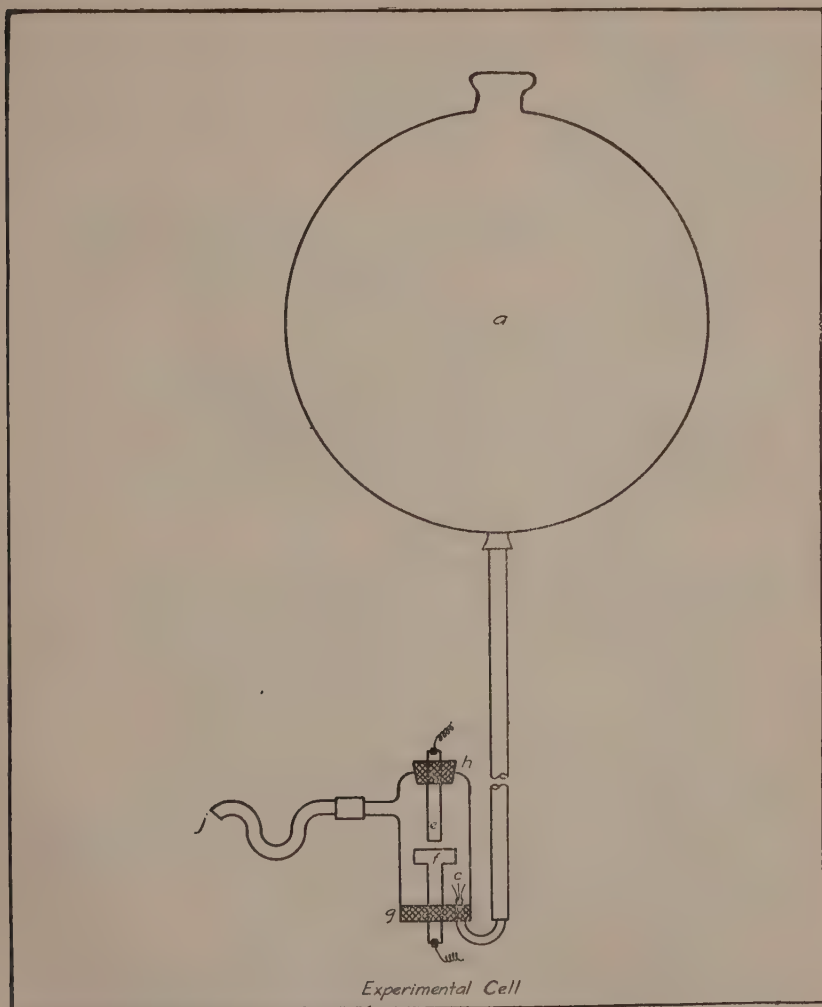


Fig. 1. Experimental outfit.

Several other electrode materials were then tested. Nichrome electrodes decomposed, forming a black precipitate. Tantalum electrodes were also tried. Since tantalum will pass a current only in one direction, it was used as a cathode. A carbon electrode was used as an anode. The tantalum electrode disintegrated so rapidly that it was found to be impractical. Carbon electrodes were next tried in the cell. At first some trouble was caused by a dark colloidal precipitate which discolored the solution. After running for two hours this discoloration stopped; from then on no trouble

FIGURE II  
Automatic Apparatus.



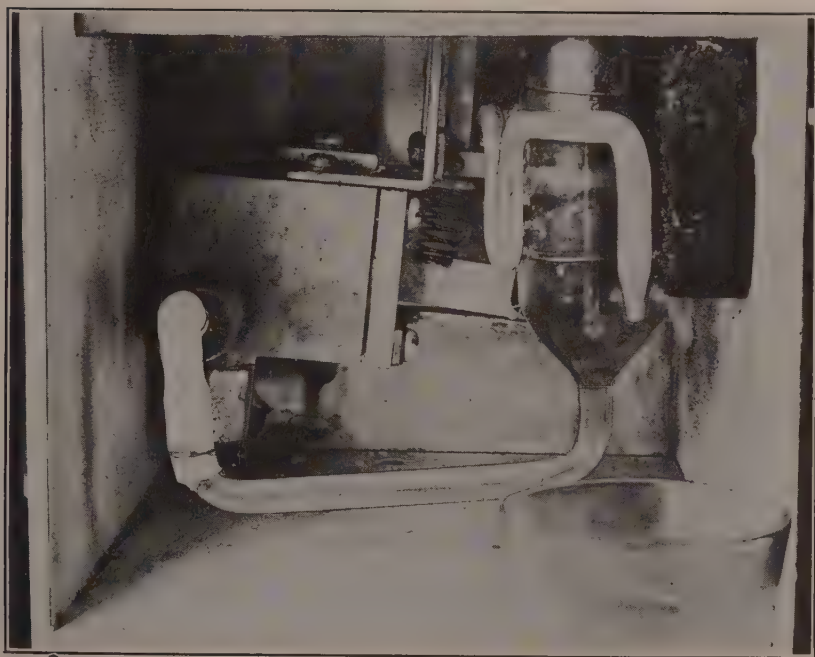
FIGURE II



## FIGURE III

Details of Valve and Cell Construction.

FIGURE III





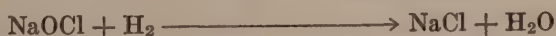


was experienced. The black precipitate was apparently due to impurities present in the carbon, which had been taken from an old dry cell.

The apparatus for preparing Dakin's solution shown in Figure 1 was constructed. A saturated solution of salt was prepared by allowing distilled water to filter through a bed of sodium chloride in the large glass funnel, *a*. The saturated salt solution ran through a rubber tube to the cell. A screw clamp was used to open and close the flow. At *b* a piece of glass tubing drawn to a capillary opening controlled the flow of the salt solution. The cell was made from one end of the jacket of a water condenser. The salt solution entered at *c*. The electrodes, which are of carbon, are at *e* and *f*, *f* being the anode and *e* the cathode. The rubber stoppers *g* and *h* hold the electrodes in place. The outlet for the liquid is at *j*. Current was supplied by a storage battery, which was charged by a battery charger. The current was controlled by a slide wire resistance and determined by an ammeter.

By placing the anode at the bottom of the cell the chlorine gas has a good chance to react with the sodium hydroxide that is formed at the cathode. The rate of flow is slow enough for this reaction to take place.

The hydrogen evolved collects in the top of the cell. The increasing pressure of the hydrogen tends to force the liquid out intermittently. Due to the presence of the hydrogen the cell should be colored to keep out sunlight. Hydrogen and chlorine combine in sunlight with explosive violence to form hydrogen chloride. Hydrogen also reacts with sodium hypochlorite as follows:



The hydrogen was taken off through a glass tube surrounding the cathode. There was no change in the concentration of the hypochlorite formed so that the reaction apparently did not occur to any appreciable extent. The reaction occurs only with nascent hydrogen so it is desirable to maintain a high current density at the cathode. The hydrogen comes off with the solution and because of its high diffusion rate should cause better penetration of the wound. It is advisable to keep the NaOCl away from the anode as the hypochlorite is more readily electrolyzed than sodium chloride forming the chlorate. While the chlorate does no harm in the solution it is wasteful of current. A high current density at the anode tends to keep the hypochlorite ions away.

The experimental results produced by this cell are tabulated in Table 2. From these results it was decided that the rate of flow through the cell should be 6 c.c. per minute when using 1.4 amperes.

The improved automatic outfit shown in Figures 2 and 3 was designed and built. The new cell used carbon electrodes of the design shown in Fig. 4. The anode was turned from a one-inch carbon rod in a lathe. The cathode was made with a hole to slip over the upper part of the anode. The space between was filled with insulating material. Several materials were tried. Sealing wax had been shown to be unsatisfactory on the previous electrodes. Tar was objectionable because of the yellow color it imparted to the solution. Paraffin melted after the cell had run awhile. The best results were obtained with mica and De Khotinsky cement. The anode rod was covered with several thin layers of mica and

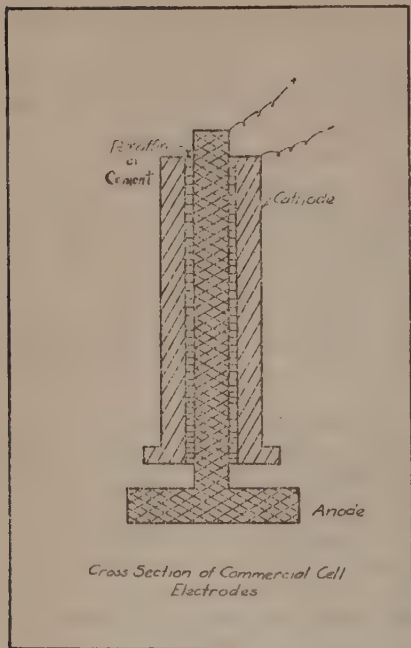


Fig. 4. Cross section of commercial cell electrodes.

rent to the cell was turned on the valve would open and allow the salt solution to flow to the cell. With the current and flow of liquid synchronized it was only necessary to turn one switch in order to start the apparatus to working. All parts of the apparatus except the salt saturator may be placed in a container approximately 6 by 12 by 7 inches.

Tests on this cell showed that with a current of 1.4 amperes and a flow of 6 c.c. per minutes that after the first ten minutes of operation the concentration of Dakin's solution was well within the desired limits. Tests for alkalinity with dry phenolphthalein showed no signs of sodium hydroxide. The data from these tests is given in Table 2.

Another series of runs was made with a constant rate of flow but with varying current so as to show the relation between concentration and current. These data are given in Figure 5. It accounts for the low concentration.

## CONCLUSION

An automatic electrolytic apparatus for the production of Dakin's solution of the proper concentration and pH value for surgical use has been designed, constructed and tested. In the solution produced, both boric acid and sodium bicarbonate have been left out since these are not necessary to adjust the pH value. The apparatus described will enable the surgeon to have Dakin's solution of the approved concentration by

forced into the cathode. Hot cement was then poured in between the two electrodes. The cell container was made entirely of glass with an inlet for the salt solution at the bottom. The side arm outlet contained a U bend to insure complete absorption of the chlorine gas. The top of the cell was open to take the electrodes, which were sealed in a rubber stopper.

The cell, without electrodes and with outlet arm closed, held 75 c.c. of the liquid. Below the outlet arm the capacity is 50 c.c. without electrodes and 37 c.c. with the electrodes inserted.

Current was supplied directly by a Balkite rectifying unit without using the storage battery. A dry charging unit would be more convenient. The current was controlled by a rheostat. The rate of flow of the saturated solution from the salt reservoir was controlled by a glass pet cock. The salt solution was also passed through an automatic valve connected in such a manner that when the current

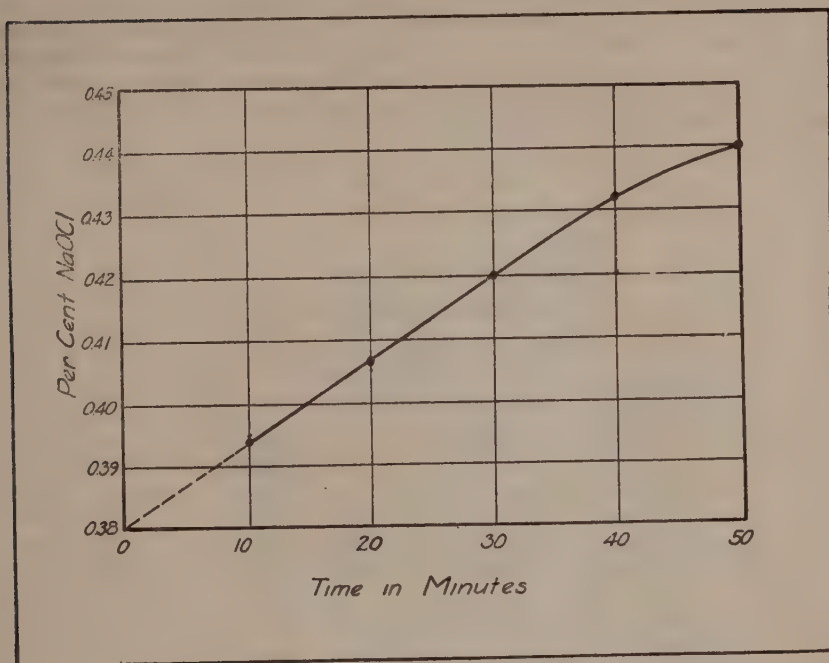


Fig. 5. Relation of time and sodium hypochlorite production.

merely turning a switch. The apparatus used experimentally is adequate for office and portable use. Larger apparatus can be designed for hospital use.

There are various ways of using the cell. In case of an accident, for example, when the patient is brought in the machine could be started and the solution used for sterilizing the doctors' and nurses' hands. The solution could then be allowed to drip onto the wound. If desired, the solution could be allowed to run onto the wound while the operation was in progress. The open wound could be irrigated by allowing the solution to drip on it as long as desired. During the World War Dakin's solution was allowed to drip onto wounds for as long as 48 hours.

In the home a small cell could be wired in permanently. A portable set could be carried by a physician in his car. It could be connected directly to the regular house lighting system. Where no electricity was available the cell could be operated directly from the storage battery of the physician's car by the installation of suitable wiring. If it were desired to leave it in the home it could be connected to a car or radio storage battery.

For some purposes it might be desirable to produce Dakin's solution containing enough sodium chloride to make it isotonic with blood serum. An isotonic sodium chloride solution has a concentration of approximately 0.9 per cent sodium chloride. It should be possible to supply the cell with sodium chloride solution of such a concentration that it would deliver sodium hypochlorite of the proper concentration with sufficient sodium



chloride to give an isotonic solution. If the ionization of sodium hypochlorite were the same as sodium chloride a 0.9 per cent solution of sodium chloride would electrolyze to form the desired solution. Due to differences in ionization, however, the production of the isotonic solution should be studied experimentally. Since this is not a Chemical Engineering problem, it has not been studied in this laboratory.

TABLE 1. SUMMARY OF DATA ON PRELIMINARY RUNS

Run	Time hrs.	Temp. °C.	Current Amperes	Voltage Volts	NaOCl Grams	Sol'n Grams	NaOCl %	Na <sub>2</sub> CrO <sub>4</sub> % of NaCl	NaOH %	H <sub>2</sub> BO <sub>3</sub> g/100 c.c.
A	2	25	.43	3.3	.815	455	.179	0	-----	0
B	2	0.5	.47	3.3	.440	126	.349	0	-----	0
C	1½	0.5	.44	3.3	.268	48	.558	1	-----	0
D	2	25	.60	3.3	.802	132	.608	1	-----	0
E	2	0.5	.53	3.3	1.01	132	.77	0.4	-----	0
F	1	25	.58	3.3	.169	312	.0543	0	1.76	0
G	1	25	.58	3.3	.067	96	.0698	0	1.25	.0913
H	1	25	.93	3.3	.198	84	.236	0	1.43	.450
I.a	1	25	.64	3.3	.164	108	.152	0	1.513	0
I.b	1	25	.83	3.3	.436	132	.331	0	1.513	0
I.c	1	25	.93	3.3	.291	126	.231	0	1.510	0
I.d	1	25	1.06	3.3	.239	120	.2441	0	1.65	0
J	1	25	1.01	3.3	.358	108	.3316	0	1.513	0
K.a	1	25	.98	3.3	.340	102	.333	0	1.515	0
K.b	1	25	.98	3.3	.340	102	.333	0	0	.703

TABLE 2. RELATION OF RATE OF FLOW AND CURRENT DENSITY TO CONCENTRATION OF SODIUM HYPOCHLORITE

No.	Rate of Flow c.c. per min.	Time in minutes	Current in amperes	Percent NaOCl
A1	12	0	2.0	0.3560
A2	12	30	2.0	0.3650
A3	12	60	2.0	0.3600
B1	6	0	1.8	0.5700
B2	6	30	1.7	0.5870
B3	6	60	1.8	0.5790
C1	6	0	1.4	0.3818
C2	6	30	1.4	0.4615
C3	6	60	1.4	0.4715
C4	6	90	1.5	0.4950
C5	6	120	1.4	0.4656

TABLE 3. CONCENTRATION OF SODIUM HYPOCHLORITE WITH CONSTANT FLOW AND CURRENT

Rate of flow 6 c.c. per minutes. Current  
1.4 amperes

Time in minutes	Per cent NaOCl
10.....	0.3940
20.....	0.4060
30.....	0.4197
40.....	0.4320
50.....	0.4360

# STUDIES ON THE INSECT FAUNA OF IOWA PRAIRIES

GEORGE O. HENDRICKSON\*

*From the Department of Entomology, Iowa State College*

Accepted for publication Oct. 16, 1929

## ACKNOWLEDGMENTS

The author is deeply indebted to Dr. H. H. Knight, under whose direction the work was carried on, for valued suggestions and ready assistance, to Dr. C. J. Drake for encouragement and advice, to Dr. W. H. Wellhouse for helpful criticisms, to Mr. G. C. Decker for reciprocal assistance in collecting and rearing a number of specimens, to Dr. L. H. Pammel for aid in plant identification and Dr. J. H. Aikman for advice in the interpretation of the plant communities. The following systematists have been of invaluable assistance with their determinations of species of insects: Dr. J. W. Folsom (*Collembola*), Dr. B. B. Fulton (*Orthoptera*), Mr. Nathan Banks (*Neuroptera*), Mr. G. S. Walley (*Ephemera*, *Chironomidae*, *Scutelleridae*, *Homoptera*), Mr. B. E. Montgomery (*Odonata*), Dr. P. W. Claassen (*Plecoptera*), Dr. J. D. Hood (*Thysanoptera*), Dr. H. H. Knight (*Hemiptera*), Dr. C. J. Drake (*Tingitidae*), Dr. H. M. Harris (*Nabidae*, *Saldidae*), Mr. H. G. Barber (*Pentatomidae*, *Neididae*, *Lygaeidae*), Dr. E. D. Ball (*Homoptera*), Dr. D. W. DeLong (*Homoptera*), Dr. P. B. Lawson (*Homoptera*), Mr. W. T. Davis (*Cicadidae*), Dr. F. C. Hottes (*Aphididae*), Prof. H. F. Wickham (*Coleoptera*), Mr. W. J. Brown (*Coleoptera*), Mr. N. K. Bigelow, assisted by Mr. Chas. W. Leng (*Coleoptera*), Mr. Chas. Schaeffer (*Coleoptera*), Mr. K. F. Chamberlain (*Chrysomelidae*), Dr. G. M. Stirrett (*Chrysomelidae*), Mr. M. C. Lane (*Carabidae*, *Elateridae*), Dr. L. L. Buchanan (*Rhynchophora*), Dr. W. T. M. Forbes (*Lepidoptera*), Mr. Carl Heinrich (*Lepidoptera*), Mr. August Busek (*Lepidoptera*), Mr. W. Schaus (*Lepidoptera*), Dr. C. Betten (*Trichoptera*), Prof. J. S. Hine (*Diptera*), Dr. J. M. Aldrich (*Diptera*), Dr. C. H. Curran (*Diptera*), Mr. C. T. Greene (*Diptera*), Dr. O. A. Johannssen (*Chironomidae*), Dr. Robt. Matheson (*Culicidae*), Dr. C. P. Alexander (*Tipuloidea*), Dr. C. L. Fluke (*Syrphidae*), Mr. H. W. Allen (*Tachinidae*), Dr. D. G. Hall (*Sarcophagidae*), Dr. H. C. Hockett (*Anthomyiidae*, *Muscidae*), Miss Grace Sandhouse (*Hymenoptera*), Mr. S. A. Rohwer (*Hymenoptera*), Mr. R. A. Cushman (*Hymenoptera*), Mr. A. B. Gahan (*Hymenoptera*), Mr. L. H. Weld (*Hymenoptera*), Mr. W. M. Mann (*Formicidae*), Dr. M. R. Smith (*Formicidae*), Dr. H. H. Ross (*Tenthredinidae*), Dr. T. H. Frison (*Bombidae*), and Dr. Harold Morrison, National Museum, who directed the work of identification in several orders.

---

\*A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, major subject Entomology.

## INTRODUCTION

Seven-eighths of the area of the State of Iowa was once covered with prairie (Shimek, 1911). But most of the native prairie has rapidly disappeared before the army of homebuilders. The remaining prairie is restricted largely to railroad rights-of-way, roadsides, rural school grounds and State Parks. A few privately owned larger areas whose roughness, poor soil, or drainage problems have saved them from the breaking plow, exhibit typical prairie flora. The poorly drained tracts realize such net profits to their owners, that it seems practical in many cases to leave them in permanent meadows for hay. The rough land is generally pastured, but in most cases not heavily. Many smaller patches of prairie flora along railroad rights-of-way, roadsides and on rural school grounds will probably persist for a long time. The State Park plans show provision for the retention of natural conditions, and will probably be expanded before long to include more extensive areas of native grassland.

The small amount of prairie which remains presents insect problems which are worthy of study. Children of the rural schools meet with numbers of prairie insects on their way to school and on the school grounds. The rural teachers are in need of assistance in the determination of these insects, whose interesting habits are observed by the pupils. Every year these areas of native vegetation show some centers of breeding increased numbers of certain insect pests, which spread out and infest neighboring cultivated crops. These virgin tracts are of great interest and value to the collector and taxonomist who find these places accessible for the collection and observation of many species. Ecological and physiological studies with the insects and the original flora, may furnish data and principles for comparative study in the probable distribution and the control of injurious insects. Because of the general interest to all of its citizens in the original conditions of a state, records of the fauna of native prairie at various intervals of time add to the history of the state. These needs and interests suggested that a general survey of the insect fauna of some of the larger tracts of prairie in the several parts of Iowa, together with the associations of the species with plant communities, would be of value.

## REVIEW OF LITERATURE

Earlier investigators have supplied us with several lists of species of the major orders of insects of Iowa. Lists of the insect fauna of a few counties have been published. A large number of papers have dealt with the life histories and habits of insects of the state. But the attention of the author has not been directed to any detailed associational study of the insect fauna of Iowa prairies.

Allen (1871) dealt with associational distribution of the fauna of prairies in a general way. Concerning insects he wrote: "The insect fauna presents peculiarities similar to those of the flora, on which their existence is so intimately depended. Certain groups are represented in an unusual variety of species and abundance of individuals, but the most numerous forms are often exceedingly localized. Other groups are again but sparsely represented. No country, however, it is to be hoped, is richer in Orthoptera (grasshoppers), either in species or individuals; and a few species of butterflies are also especially numerous, of which a small portion seems to



be strictly prairie forms. The Hemiptera and Neuroptera exist in great abundance, the dragon flies being richly represented, both as respects the number of species and the gorgeousness of their colors, many of which are never or rarely seen in the Atlantic States. The Hymenoptera, on the other hand, are comparatively few, especially the bees and wasps, notwithstanding the abundance of the flowers. If the Diptera, however, do not make up the equilibrium, it is not because mosquitoes and blood-sucking flies (Tabanidae) are deficient either in variety of species, in number of individuals, in size or in voracity."

Brumfiel (1919), on the rolling prairies of Johnson county, Iowa, found "the highly specialized orders of Hymenoptera, Diptera, Lepidoptera and Coleoptera were abundant" in species and numbers, and on the sand-dune prairies of the same county that the "Tiger beetles and digger wasps . . . " were the most common invertebrates.

The prairie fauna of the neighboring state of Illinois has been studied quite intensively in recent years. Shelford (1913) surveyed the prairie communities in the region about Chicago. He listed 56 species of insects from low prairie and temporary marshes, and 31 species from compass-plant (*Silphium*) prairie. Vestal (1913) found 350 species of animals on sand prairie and grouped them with several plant communities. Adams (1915) extended his studies over several types of prairie in several parts of the state. He listed 130 species of prairie insects, and through reference to previous literature and through his own observations, showed that many of these insects are associated with the grasslands. However, his lists of characteristic insects of each of several plant communities are small. Shackelford (1929) made quantitative studies of several plant communities of Illinois prairie, and has given names to two animal communities. For the animal community associated with the *Andropogon* associates of plants she has originated the name of *Lygas-Formica-Microtus* Prescoecies. To the animal community associated with the low prairie (*Carex* and *Salix longifolia*) the name of *Cambarus-Eucrangonyx* Prescoecies was given. Approximately 150 animals were taken as members of each Prescoecies, and about 100 of these in each case are insects.

For Kansas, Hayes (1927) stated that "the Hemiptera, represented by such abundant species as the tarnished plant-bug (*Lygus pratensis* Linn.) and other leaf and plant bugs, is perhaps the predominant order of insects of the prairie." He found the scavenger group of insects to be one of the interesting groups. The seasonal abundance of the chinch-bug in prairie grasses attracted his attention. Whelan (1927) surveyed the winter fauna of species of bunch grass (*Andropogon* spp.) of eastern Kansas and found that *Collembola* composed about 25 per cent of the animal life, while chinch bugs were greater in number than any other species, as they constituted about 35 per cent, 7 per cent of these being dead. The ants composed 4 per cent, the click beetles 2 per cent and the snout beetles 1 per cent. Of the remaining per cent, most of the insects were contained in the two orders *Hemiptera* and *Coleoptera*. After a brief discussion on the economic relations of these insects the following conclusion was made, "From the above data, however, we conclude that if the inhabitants of the bunch-grasses were all killed when their hibernating places were burned, there can be little doubt but that many more injurious insects than beneficial ones would be killed."



## COLLECTING STATIONS

The author's study of the prairie began several years ago with the flora and the types of native grasslands in the state. The following outline of the general types of the prairie of Iowa by Shimek (1911), which came into the hands of the author early in his work, suggested places for investigation. "The areas which were originally covered with prairie flora are divided into these six more or less distinct types:

1. The broad, flat plains which characterized the Wisconsin and Iowa drift areas and a part of the uneroded Kansan drift area such as may be observed in Osceola county and southward.
2. The more rolling drift surfaces are presented by the greater part of the Kansan area and the more or less distinct moraines bordering the Wisconsin and Iowa areas.
3. The very rough loess ridges which border the Missouri valley and which present the most extreme xerophytic conditions in Iowa. Similar conditions are found in the rough Wisconsin morainic (?) region in southwestern Lyon county, and the floras of the two areas are practically identical.
4. The well-drained alluvial plains such as are shown at their best along the Missouri, but which are more or less developed along all streams.
5. The prairie ridges which appear in all the forested rougher parts of the state, but are most striking in the more heavily timbered eastern parts where they have been known as 'oak openings' because the surrounding forest, consisting largely of oaks, encroached upon them.
6. The sand-dune areas. These are usually considered distinct from the prairie, but a comparison of the floras shows that they differ very little."

The knowledge of exact locations of tracts of virgin land came from various sources other than the author's personal observations. Several extension service and research workers of Iowa State College furnished approximate information. In several instances conversations with older settlers in a number of communities aided materially. Bankers in several smaller towns of central and northern Iowa were able to direct the author to some of the best tracts. More than one hundred tracts varying in size from one to several thousand acres were seen. These areas were more numerous in the north, central and western parts of the state.

All of the observed areas showed evidence of disturbance. The western hillsides along the Missouri river were grazed, but only those which showed the least disturbance were studied. Most of the northern and central tracts were mown by September, yearly. Parts which showed the dominance of blue grass (*Poa pratensis* L.), of red top (*Agrostis alba* L.), or other marked disturbance at any season of the year, were not studied. Unknown smaller tracts were grazed at least during a part of the year.

The least disturbed prairie was seen 6 miles west and 1.75 miles north of Thompson. There, an area of one hundred and sixty acres with low hills and kettle holes showed little disturbance except along the south and west sides, where wagon traffic had brought about conditions that permitted blue grass (*Poa pratensis*) to gain some dominance.

The studies were made at forty different locations which exhibited varying communities of plants. When each area was reached, notes were taken on the plants in the portions from which insect collections were to be made. These notes listed the dominant grasses, and more common herbaceous plants with their relative abundance. Most of the plants were identified in the field and the scientific names according to Gray (1908) were used. Later the plant communities were classified according to a general plan outlined by Clements (1920). The following list of stations is fitted into the plan, and sub-divided to coincide with the centers of insect collection:

Grassland Climax (*Stipa*—*Bouteloua* Formation)

A. True Prairie (*Stipa*—*Koeleria* Association)

a. *Stipa spartea*—*Andropogon scoparius* association

Topography: hills, sides of hills

General location: central and northern Iowa

Stations:

Northeast of Iowa State College grounds

2 miles north of Ames

5 miles northwest of Buffalo Center

3.5 miles north of Ledyard

6 miles northwest of Ledyard

1.5 miles northeast of Ocheyedan

Ocheyedan Mound roadside

5 miles south of Stanhope

7.75 miles northwest of Thompson

b. *Andropogon scoparius*—*Bouteloua curtipendula* association

Topography: steep hillsides

General location: bluffs of Missouri and Big Sioux rivers

Stations:

4 miles northeast of Beloit

Council Bluffs

Gitchie Manito State Park

1 mile west of Hamburg State Park

1 mile north of Reels City

Sergeant Bluff

15 miles north of Sioux City

3 miles south of Westfield

1. Sub-climax prairie (*Andropogon* associates)

a. *Andropogon furcatus*—*Sorghastrum nutans* associates

Topography: hillsides near woods, river flood plains

General location: southeastern Iowa

Stations:

1 mile south of Amana

Lacey Keosauqua State Park

1.5 miles east of Muscatine

3 miles south of Muscatine

- 1 mile east of Verdi
- 6 miles south of Washington

b. *Andropogon furcatus*—*Spartina Michauxiana* associates

(1). *Andropogon furcatus* consociates

Topography: level, mostly wet meadow

General location: northern, central and western Iowa

Stations:

- 10 miles southwest of Ames
- 2.5 miles south of Ames
- 8 miles southeast of Britt
- 5 miles northwest of Buffalo Center
- 6 miles northwest of Cedar Falls
- 2 miles south of Ledyard
- 3.5 miles north of Ledyard
- 6 miles northwest of Ledyard
- 6 miles northwest of LeMars
- 5 miles east of Renwick
- 4 miles northwest of Thompson
- 7.75 miles northwest of Thompson

(2). *Spartina* consociates

Topography: low, level, wet, frequently with some standing water in spring, but not in summer

General location: several parts of the state

Stations:

- 2.5 miles north of Ames
- 2.5 miles south of Ames
- 1 mile south of Amana
- Lake Amana shore
- 8 miles southeast of Britt
- 5 miles northwest of Buffalo Center
- 6 miles northwest of Cedar Falls
- Gitchie Manito State Park
- 1 mile southeast of Gruver
- 2 miles southwest of Kelso
- 10 miles southwest of Kelso
- 4 miles north of Le Mars
- 3.5 miles north of Ledyard
- 6 miles northwest of Ledyard
- .5 mile south of Missouri Valley
- 5 miles east of Renwick
- 4 miles northwest of Thompson
- 7.75 miles northwest of Thompson
- 6 miles south of Washington

(3). *Carex* sociates

Topography: low, wet, often with standing water in the spring, but not in summer

General location: several parts of the state

Stations:

- 1 mile south of Amana
- Lake Amana shore
- 2.5 miles north of Ames
- 2.5 miles south of Ames
- 8 miles southeast of Britt
- 5 miles northwest of Buffalo Center
- 6 miles northwest of Cedar Falls
- 3.5 miles north of Ledyard
- 4 miles northwest of Thompson
- 7.75 miles northwest of Thompson

(4). *Polygonum amphibium* socius

Topography: low, wet, with standing water in spring, but not in summer

General location: several parts of the state

Stations:

- 2.5 miles south of Ames
- 1 mile south of Amana
- 5 miles northwest of Buffalo Center
- 10 miles southwest of Kelso
- 3.5 miles north of Ledyard
- .5 mile south of Missouri Valley
- 2 miles west of Pacific Junction
- 7.75 miles northwest of Thompson

#### B. Mixed Prairie (*Stipa*—*Bouteloua* association)

##### 1. *Bouteloua hirsuta*—*B. curtipendula* association

Topography: gravelly hilltops

General location: central and northern Iowa

Stations:

- 2 miles north of Ames
- Ocheyedan Mound
- 1.5 miles northeast of Ocheyedan
- 5 miles south of Stanhope

The list of plant communities is not complete. Only the more prevalent and conspicuous groupings were studied. The catalogue of grasses of Iowa by Pammel, Ball and Scribner (1903) aided the author in making and in checking his findings. The list of prairie flora by Shimek (1911), which shows the relative distribution and occurrence of several hundred species of plants on the several types of prairie, was of great service in checking the communities. Hayden (1919) recorded the floristic features of a prairie province in terms of ecological communities. This province, two miles north of Ames, was studied intensively by the author to partially guide his observations at other stations.

Some of the communities such as the *Bouteloua hirsuta*—*B. curtipendula* association and the *Stipa spartea*—*Andropogon scoparius* association were no longer extensive in area because of the general tillage and pasturing of such lands in the state. The communities on lower and rougher



ground were more numerous and extensive. Other communities such as *Buchloe dactyloides*—*Bouteloua hirsuta* association of Gitchie-Manito State Park may occur, but they were not seen in tracts of sufficient extent to permit of the collection of typical insects from them.

#### METHODS OF COLLECTING INSECTS

Several stations within one hour's driving distance of Ames were visited one to several times each week from about the first of March until November, or until mown in the later part of August, during the years 1925 to 1928, inclusive. At these stations basic collections were made at most of the plant communities of this study. Except at the most distant situations collections were made at other stations each month from May to October during one or more years. The extreme western and southeastern stations were visited during July and August of 1928.

Because an insect net was the chief means of collecting most of the specimens were adults. Larvae and nymphs occurring on the more common plants were brought in to be reared in the insectary, unless they could be readily recognized without rearing. From some of these immature stages a few parasitic insects were obtained. Some time was taken regularly at each station for the collection of insects on the ground. Stones and debris were frequently turned over in the search of specimens. Because permission from the owner of a tract usually carried with it admonition against digging up plants, little study was made of insects of the soil, except at the *Spartina* consocieties near Gruver, where permission to dig was readily obtained.

In securing data concerning the host plants and inhabited communities, the larger groups of single species of plants and the several communities were collected from and observed separately. These separate collections were placed in individual killing bottles in the field and the numbers of the bottles were associated with notes on the plants. Later, when the insects were mounted, small numbers plus the locality labels served to key the data concerning plants with the insects.

Relative quantitative data were secured in several ways. At each sweeping a specimen of each different species was taken and prepared for permanent preservation until the author became acquainted with some of the widely distributed common forms. After that those forms were counted but not preserved unless a specimen showed some variation from the general appearance of the species. All specimens of rare species were saved. In this manner the insects of each community were secured in approximately correct relative ratios. The visits to several stations of each of the plant communities increased the number of species as well as aided in settling the questions of relative occurrence and most frequented communities of the various species. The general and extensive collecting made possible a survey of more communities in several parts of the state.

#### DETERMINATION OF INSECT SPECIES

Except for a few commonly known insects, all determinations for the many families and orders of insects have been made by recognized specialists. Identification of specimens by comparison with those determined by spe-

cialists was attempted only in a few species. Many of the specimens will be turned over to the collection of the Department of Zoology and Entomology, Iowa State College. To increase the value of this survey, the services of specialists were sought and deemed requisite to thorough work. It became apparent to the author early in the problem that neatly mounted specimens with locality labels and in large series were appreciated by many specialists. The more extensive collecting brought out a few new species, which aided considerably in securing the services of specialists.

#### ANNOTATED LIST

The following list furnishes the names of the species as given by the specialists whose names occur at the head of each order. The orders are arranged according to Comstock (1924). Within an order the species are arranged for the most part in accord with the most recent catalog of the order or some general list of insects. In several orders approximate positions of several species were secured from other sources, such as systematic papers and correspondence with specialists who determined the species in question. The chief work consulted in the arrangement of the species of an order is given at the head of the list of that order.

The plant community, or communities, at which each of the more generally distributed and numerous insect species occurred, is given with dates of the earliest and latest collections of adult forms. In the species which were observed or collected in less numbers, the dates and localities of collection are given. If the food habits of an insect were observed, the record occurs with the species in the list. When the number of individuals of a species taken or seen at a community was five or more during one season, the word common is applied frequently unless the number became so large that the word numerous could be used properly.

#### ORDER COLLEMBOLA

The species were determined by Dr. J. W. Folsom. The arrangement follows Guthrie (1903).

##### *Orchesella ainsliei* Fol.

At *Andropogon furcatus* consocieties under dead grass and moss, 2.5 mi. south of Ames, Sept. 22, 1928, two specimens.

##### *Isotoma viridis* Bour.

At *Andropogon furcatus* consocieties under dead grass and moss, 2.5 mi. south of Ames, Sept. 22, 1928, one specimen.

##### *Entomobrya purpurascens* Pack.

At *Stipa spartea*—*Andropogon scoparius* association, under stones, 5 mi. south of Stanhope, Oct. 6, 1928. Numerous. At *Andropogon furcatus* consocieties, under stone, 7.75 mi. northwest of Thompson, one specimen, Sept. 15, 1928.

##### *Lepidocyrtus cyaneus* Tull.

At *Andropogon furcatus* consocieties, under stone, 7.75 mi. northwest of Thompson, Sept. 15, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, under stone, 2 mi. north of Ames, Aug. 16, 1928, three specimens.

*Lepidocyrtus* sp.

At *Andropogon furcatus* consocieties under stone, 7.75 mi. northwest of Thompson, Sept. 15, 1928, one specimen.

## ORDER ORTHOPTERA

The species were determined by Dr. B. B. Fulton. The arrangement follows Blatchley (1920) and Scudder (1900).

*Litaneutria minor* ? Scudd.

One specimen, a nymph, at *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928. A second nymph eluded capture. No others were seen.

*Diapheromera veliei* Walsh

At all *Andropogon* communities. Earliest adult, July 10, 1925. Latest adult, Sept. 19, 1928. Numerous in some places.

*Acrydium ornatum* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, March 25, 1928; latest adult, Sept. 15, 1928. Scattered over all of associates in spring. Restricted more to *Spartina* consocieties after May 1, when temporary ponds had usually disappeared. The most common grouse locust.

*Paratettix cucullatus* Burm.

One adult specimen, May 30, 1926, 2.5 mi. south of Ames, at *Andropogon furcatus* consocieties.

*Tettigidea lateralis parvipennis* Harr.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, March 23, 1926; latest adult, Aug. 14, 1926. Most numerous at *Spartina* consocieties.

*Pseudopomala brachyptera* Scudd.

At *Andropogon scoparius*—*Bouteloua curtipendula* association stations from Gitchie Manito State Park to 1 mi. west of Hamburg State Park, July 24-30, 1928.

*Mermiria neomexicana* Thom.

One specimen, a nymph, at *Andropogon scoparius*—*Bouteloua curtipendula* association, July 31, 1928.

*Mermiria maculipennis macclungi* Rehn

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. north of Reels City to 1 mi. west of Hamburg State Park, July 30-Aug. 1, 1928.

*Eritettix simplex* Scudd.

At *Bouteloua hirsuta*—*B. curtipendula* association, 5 mi. south of Stanhope, April 25, May 9, 1928. The author (1928) records it from a *Stipa-Bouteloua* community.

*Orphulella speciosa* Seudd.

At all *Andropogon* communities. Earliest adult, July 7, 1928, 6 mi. northwest of Ledyard; latest adult, Sept. 19, 1928, 5 mi. south of Stanhope. Most numerous at *Andropogon furcatus* consocieties and *Stipa spartea*—*Andropogon scoparius* association.

*Dichromorpha viridis* Seudd.

At *Spartina* consocieties, Lake Amana, Aug. 12, 1927, 10 mi. southwest of Kelso, July 30, 1928, and Pacific Junction, July 31, 1928. Scarce.

*Chorthippus curtipennis* Harr.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, June 26, 1926; latest adult, Aug. 26, 1926. Most numerous at *Andropogon furcatus* consocieties.

*Ageneotettix deorum* Seudd.

At all *Andropogon scoparius*—*Bouteloua curtipendula* association stations, July 24–Aug. 1, 1928. At *Bouteloua hirsuta*—*B. curtipendula* association, Ocheyedun Mound, July 23, 1928, and 2 mi. north of Ames, July 2–Aug. 7, 1926. Numerous at *Andropogon scoparius*—*Bouteloua curtipendula* association.

*Hadrotettix trifasciatus* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Dissosteira carolina* Linn.

Seen along paths and at barren places at *Andropogon scoparius*—*Bouteloua curtipendula* association, *Bouteloua hirsuta*—*B. curtipendula* association, and *Stipa spartea*—*Andropogon scoparius* association, July–October. Not numerous.

*Mestobregma kiowa kiowa* Thom.

At *Bouteloua hirsuta*—*B. curtipendula* association, Ocheyedun Mound, July 23, 1928, and *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928. Not numerous.

*Arphia pseudonietana* Thom.

At *Stipa spartea*—*Andropogon scoparius* association, 6 mi. northwest of Ledyard, one specimen.

*Chortophaga viridifasciata* DeG.

At *Andropogon furcatus* associates, and *Stipa spartea*—*Andropogon scoparius* association. The author (1928) reported earliest adult, April 21; latest adults, May 28, 1926. Nymphs found as early as March 10, and as late as Oct. 20. Not numerous.

*Encoptolophus sordidus* Burm.

At *Andropogon furcatus* consocieties, and *Stipa spartea*—*Andropogon scoparius* association. The author (1928) reports earliest adult, Aug. 7; latest adult, Oct. 1, 1926. Not numerous in any community, but probably



occurs in largest number at *Bouteloua hirsuta*—*B. curtipendula* association as reported by the author (1928).

*Hippiscus apiculatus* Harr.

Not met with since report by author (1928) when taken as adult May 15-June 4, 1926. Probably characteristic of *Bouteloua hirsuta*—*B. curtipendula* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association, but not numerous.

*Hippiscus haldemanii* Scudd.

At *Stipa spartea*—*Andropogon scoparius* association, northeast of Iowa State College grounds, July 11, 1928. Rare.

*Hesperotettix pratensis* Scudd.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park to Sergeant Bluff, July 24-26, 1928. Not numerous.

*Hesperotettix speciosa* Scudd.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, one specimen.

*Schistocerca alutacea* ? Harr.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, and Sergeant Bluff, July 26, 1928. All specimens are nymphs.

*Melanoplus dawsoni* Scudd.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, *Bouteloua hirsuta*—*B. curtipendula* association, and *Stipa spartea*—*Andropogon scoparius* association. The author (1928) records this species at *Stipa*—*Bouteloua* community with July 15 as earliest date of collection of adult, and Oct. 9, the latest date.

*Melanoplus scudderi* ? Uhl.

One specimen at *Polygonum* sociies, 10 mi. southwest of Kelso, July 30, 1928.

*Melanoplus mexicanis atlanis* Riley

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park to Council Bluffs, July 24-Aug. 1, 1928, and at *Andropogon furcatus* consociies, 2.5 mi. south of Ames, June 23 and Aug. 26, 1926. More numerous in western communities.

*Melanoplus femur-rubrum* DeG.

At all *Andropogon* communities. Earliest adult, July 26, 1928; latest adult, Aug. 24, 1928. Most numerous at *Andropogon furcatus* consociies.

*Melanoplus packardii* Scudd.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City to 1 mi. west of Hamburg State Park, July 26 to July 31, 1928. Scarce.

*Melanoplus confusus* Scudd.

At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, July 7, 1928, 2 specimens; at *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, one specimen. The author (1928) took this species at *Stipa*—*Bouteloua* community, May 3-Aug. 14, 1926. Only a few specimens have been secured.

*Melanoplus keeleri luridus* Dodge

At all *Andropogon* communities. Earliest adult, June 23, 1928; latest adult, Oct. 20, 1928. Most numerous at *Stipa spartea*—*Andropogon scoparius* association and *Andropogon scoparius*—*Bouteloua curtipendula* association.

*Melanoplus differentialis* Thom.

At *Andropogon furcatus*—*Spartina Michauxiana* associates where tall herbs such as *Helianthus grosseserratus* occur. Earliest adult, Aug. 20, 1927; latest adult, Oct. 20, 1928. Not numerous.

*Melanoplus bivittatus* Say

At all *Andropogon* communities. Earliest adult, July 24, 1928; latest adult, Aug. 5, 1927. Not numerous.

*Phoetaliotes nebrascensis* Thom.

The author (1928) records this species from a *Stipa*—*Bouteloua* community, and it is probably most numerous at the *Bouteloua hirsuta*—*B. curtipendula* association.

*Arethaea gracilipes constricta* Brunn.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, quite common.

*Scudderella texensis* S. P.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, July 20, 1925; latest adult, Aug. 13, 1927. Probably most frequently met at *Spartina* consocieties.

*Scudderella pistillata* Brunn.

At *Andropogon furcatus* consocieties, 6 mi. northwest of Le Mars, July 26, 1928, two specimens.

*Scudderella furcata* Brunn.

At *Carex* societies, 1 mi. south of Amana, Aug. 25, 1928, two specimens, and at *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1928.

*Amblycorypha rotundifolia brachyptera* Ball

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, July 5, 1926; latest adult, Aug. 31, 1927. Not numerous. Most taken at *Andropogon* consocieties.

*Neoconocephalus ensiger* Harr.

At *Andropogon* communities. Earliest adult, July 17, 1926; latest

adult, Aug. 25, 1928. Most of the specimens were taken at *Andropogon furcatus* consocieties.

*Orchelimum vulgare* Harr.

At *Andropogon furcatus*—*Spartina Michauxiana* and *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, July 30, 1928; latest adult, Aug. 24, 1928. Most of the specimens were taken at *Spartina* consocieties.

*Orchelimum gladiator* Brunn.

At *Spartina* consocieties, 2.5 mi. north of Ames, Aug. 5, 1927, three specimens.

*Orchelimum nigripes* Seudd.

At *Andropogon furcatus*—*Spartina Michauxiana* and *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, Aug. 12, 1927; latest adult, Aug. 24, 1928. Most of the specimens were taken at *Spartina* consocieties. Not numerous.

*Orchelimum concinnum* Seudd.

At *Spartina* consocieties. Earliest adult, July 30, 1928; latest adult, Aug. 7, 1928. Not numerous.

*Conocephalus fasciatus* DeG.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, July 24, 1928; latest adult, Sept. 7, 1927. More common where grasses are taller.

*Conocephalus brevipennis* Seudd.

At *Andropogon furcatus*—*Spartina Michauxiana* and *Andropogon furcatus*—*Sorghastrum nutans* associates. Scarce.

*Conocephalus nemoralis* Seudd.

At *Spartina* consocieties, Lake Amana, Aug. 31, 1927, one specimen.

*Conocephalus strictus* Seudd.

At all *Andropogon* communities except *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, July 31, 1928; latest adult, Sept. 19, 1928. Common. Collections most numerous from *Andropogon furcatus* consocieties.

*Conocephalus attenuatus* Seudd.

At *Spartina* consocieties, Lake Amana, Aug. 12, 1927, five specimens.

*Conocephalus nigropleurus* Brunn.

At *Spartina* consocieties, 2.5 mi. north of Ames, July 16, 1928, one specimen.

*Conocephalus saltans* Seudd.

At all *Andropogon* communities. Earliest adult, July 23, 1928; latest adult, Oct. 20, 1928. Collections most numerous at *Stipa spartea*—*Andropogon scoparius* associations. Common.

*Udeopsylla nigra* Seudd.

At *Stipa spartea*—*Andropogon scoparius* association, under stones, 2 mi. north of Ames, April 25, 1927, one specimen, and 5 mi. south of Stanhope, April 25, 1928, one specimen. Both specimens are young nymphs.

*Pediodes* sp.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, one specimen.

*Nemobius fasciatus* DeG.

At all *Andropogon* communities. Earliest adult, Aug. 11, 1925; latest adult, Oct. 20, 1928. Common wherever taller plants, dead vegetation and stones provide more shelter and moisture than open situations.

*Gryllus assimilis* Fab.

At all *Andropogon* communities. Earliest adult, May 2, 1926; latest adult, Oct. 20, 1928. Quite common wherever taller plants, decaying vegetation, stones, burrows of crayfish, depressions and cracks in the soil provide hiding places and cover.

*Oecanthus nigricornis nigricornis* Walk.

At *Andropogon* communities close to wooded tracts; one specimen. 1 mi. south of Amana, Aug. 25, 1928, and two specimens, 6 mi. south of Washington, Sept. 7, 1927, and Aug. 24, 1928.

*Oecanthus nigricornis argentinus* Sauss.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. northeast of Beloit to Council Bluffs, July 25 to July 31, 1928. Not numerous.

*Oecanthus nigricornis quadripunctatus* Beut.

At all *Andropogon* communities. Earliest adult, Aug. 5, 1927; latest adult, Oct. 20, 1928. Collections are most numerous from *Stipa spartea*—*Andropogon scoparius* association.

*Anaxipha exigua* Say

At *Spartina* consociates, 10 mi. southwest of Kelso, July 30, 1928. Not numerous. Adults and nymphs collected.

## ORDER NEUROPTERA

The determinations of species were made by Mr. Nathan Banks. The arrangement follows Bank (1907).

*Hemerobius stigmaterus* Fh.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Sept. 17, 1926, one specimen.

*Micromus subanticus* Wlk.

At *Andropogon* consociates, 2.5 mi. south of Ames, May 25, 1926.



*Micromus variolosus* Hag.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Sept. 24, 1926.

*Chrysopa oculata* Say

At all plant communities. Earliest adult, June 26, 1928; latest adult, Aug. 19, 1927. A common lacewing.

*Chrysopa plorabunda* Fh.

At all communities except *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, July 6, 1928; latest adult, Aug. 24, 1928.

## ORDER EPHEMERIDA

The determinations of species were made by Mr. G. S. Walley. The arrangement of species follows Banks (1907).

*Hexagenia venusta* Eaton

At *Carex* socias, Lake Amana, June 23, 1928.

*Hexagenia* sp.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. northeast of Beloit, July 27, 1928. The Big Sioux river is about two miles west of this location. Six specimens.

*Blasturus nebulosus* Wlk.

At *Spartina* consocias, 2.5 mi. north of Ames, May 7, 1928, one specimen. Skunk River is about one-fourth mile east of this location. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, April 25, 1928. Several permanent ponds and streams occur within a mile of this location.

*Callibaetis* sp.

At *Bouteloua hirsuta*—*B. curtipendula* and *Stipa spartea*—*Andropogon scoparius* association, 1.5 mi. northeast of Ohegedan, July 27, 1928, five specimens. Rush Lake lies about one-eighth mile north of this location.

*Isonychia* sp. probably *manca* Eaton

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. northeast of Beloit, July 27, 1928, three specimens.

## ORDER ODONATA

The determinations were made by Mr. B. E. Montgomery. The arrangement follows Needham and Heywood (1929).

*Nehalennia irene* Hag.

At *Andropogon* socias, 2.5 mi. south of Ames, July 20, 1925, one female specimen.

*Enallagma hageni* Walsh

At *Spartina* consocias, 2.5 mi. south of Ames, July 3, 1926, one male specimen.

*Ischnura posita* Hag.

At *Spartina* consociés, 2.5 mi. south of Ames, two female specimens.

*Ischnura verticalis* Say

At *Spartina* consociés, 2.5 mi. south of Ames, May 20, 1927, one female specimen.

## ORDER PLECOPTERA

The species was determined by Dr. P. W. Claassen.

*Perlesta placida* Hag.

At *Carex* sociés, 1 mi. south of Amana, June 23, 1928, three specimens. The Iowa River is at the southern border of this tract.

## ORDER THYSANOPTERA

The species was determined by Dr. J. Douglas Hood.

*Frankliniella tritici* Fitch

Taken from the flower of *Ranunculus septentrionalis*, 2.5 mi. south of Ames, May 17, 1926.

## ORDER HEMIPTERA

The species were determined by Drs. H. H. Knight, C. J. Drake, H. M. Harris and Mr. H. G. Barber. The arrangement follows Van Duzee (1917), mainly.

*Homaemus aeneifrons* Say

At *Stipa spartea*—*Andropogon scoparius* association, northeast of Iowa State College campus, July 11, 1928. Swept from *Sorghastrum nutans*, 1.5 mi. east of Verdi, Sept. 5, 1928. One specimen at each location.

*Homaemus bijugis* Uhl.

At all higher *Andropogon* communities. Earliest adult, July 7, 1928; latest adult, Oct. 20, 1928. Most frequent at *Stipa spartea*—*Andropogon scoparius* association, where it is common.

*Eurygaster alternatus* Say

At *Andropogon furcatus* consociés, 2 mi. south of Ledyard, May 8, 1926, one specimen. At *Andropogon scoparius* communities, Sergeant Bluffs, July 25, 1928, one specimen, and 7.75 mi. northeast of Thompson, Aug. 6, 1928, one specimen.

*Galgupha atra* A. & S.

At *Andropogon furcatus*—*Spartina Michauxiana* associés. Earliest adult, May 20, 1927; latest adult, Aug. 13, 1927. Swept from *Elymus virginicus*, 1 mi. south of Amana, Aug. 12, 1927. Most numerous at *Spartina* consociés.

*Galgupha nitiduloides* Wolff.

At flowers of *Pedicularis canadensis*, 2.5 mi. south of Ames, May 12, 1926, two specimens. At *Andropogon furcatus* consociés, same location, May 17, 1926, one specimen.

*Corimelaena agrella* McA.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park to Sergeant Bluff, July 26-31, 1928, six specimens.

*Corimelaena lateralis* Fabr.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 1925, one specimen. Feeding on fruit of *Zizia aurea*, 1 mi. south of Amana, Aug. 12, 1927. At *Andropogon furcatus*—*Sorghastrum nutans* associates, July 20, 1928, one specimen.

*Corimelaena pulicaria* Germ.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, from flowering *Polygonum amphibium*, *Veronica virginica*, *Cicuta maculata*, most frequently. Earliest adult, May 18, 1928; latest adult, Aug. 9, 1928. Common. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928.

*Pangaeus bilineatus* Say

At *Spartina* consocieties, on ground, 10 mi. southwest of Kelso, July 30, 1928, one specimen.

*Amnestus spinifrons* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 19, 1926, one specimen.

*Amnestus pallidus* Zimm.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 20, 30, June 5, 1927, three specimens.

*Sehirus cinctus* P. B.

Swept from *Spartina* consocieties, 2.5 mi. south of Ames, July 6, 1926, and *Carex* sociies, same location, June 26, 1926. Taken at flower of *Liatis pycnostachya*, Aug. 5, 1926. One specimen in each case.

*Podops cinctipes* Say

At *Spartina* consocieties, 2.5 mi. south of Ames, May 30, 1927, one specimen.

*Sciocoris microphthalmus* Flor.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928.

*Peribalus limbolarius* Stal

At all plant communities. Earliest adult, July 11, 1928; latest adult, 5 mi. south of Stanhope, Oct. 20, 1928. On flowers of *Veronica virginica* Aug. 2, 5, 9, 1926, 2.5 mi. south of Ames. On flowers of *Solidago rigida*, 5 mi. south of Stanhope, Sept. 19, 1928. A common stinkbug, most numerous at *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon furcatus* consocieties.

*Trichopepla semivittata* Say

At flower of *Eryngium yuccifolium*, Aug. 14, 1925, 2.5 mi. south of

Ames, and at *Andropogon furcatus* consocieties, same location, Aug. 26, 1926. Two specimens.

*Trichopepla atricornis* Stal

At *Stipa spartea*—*Andropogon scoparius* association and *Andropogon furcatus*—*Spartina Michauxiana* associes. Earliest adult, July 27, 1926; latest adult, Aug. 16, 1928. On fruit of *Zizia aurea*, 2.5 mi. south of Ames, July 4, 1928, and on flower of *Erigeron ramosus*, 1 mi. south of Amana, June 23, 1928. Most frequent at *Stipa spartea*—*Andropogon scoparius* association and *Andropogon furcatus* consocieties where the species is common.

*Chlorochroa uhleri* Stal

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 26, 1928, one specimen.

*Mormidea lugens* Fabr.

At *Andropogon furcatus*—*Spartina Michauxiana* association, 2.5 mi. south of Ames, July 22, Aug. 9, 1926. Not numerous. At *Spartina* consocieties, 1 mi. south of Amana, Aug. 12, 1927, one specimen.

*Euschistus euschistoides* Voll.

At *Spartina* consocieties, 1 mi. south of Amana, Aug. 12, 1927, and at *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927. At *Carex* socies, 10 mi. southwest of Kelso, July 30, 1928. One specimen at each community.

*Euschistus variolarius* P. B.

At all communities. Earliest adult, May 15, 1926; latest adult, Oct. 20, 1928. Most numerous at *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon furcatus* consocieties. Feeding on flower of *Lepachys pinnata*, 1 mi. south of Amana, July 20, 1928. On *Astragalus caryocarpus*, 5 mi. south of Stanhope, June 15, 1928. The most common Pentatomid of the prairie.

*Coenus delius* Say

At all communities. Earliest adult, April 25, 1928; latest adult, Aug. 24, 1928. Most frequent at *Andropogon scoparius*—*Bouteloua curtipendula* and *Stipa spartea*—*Andropogon scoparius* associations. Not numerous at any community.

*Neottiglossa undata* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 14, 1926; *Spartina* consocieties 2.5 mi. south of Ames, July 11, 1928; *Spartina* consocieties, 1 mi. south of Amana, Aug. 12, 1927; *Stipa spartea*—*Andropogon scoparius* association, 3.5 mi. north of Ledyard, Aug. 7, 1928. Scarce.

*Neottiglossa sulcifrons* Stal

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park to Sergeant Bluff, July 24-26, 1928. Not numerous.

*Cosmopepla bimaculata* Thom.

At *Spartina* consocieties, 2.5 mi. north of Ames, July 16, 1928, 1 mi. south of Amana, Aug. 12, 1927, and 10 mi. southwest of Kelso, July 30, 1928.



At *Andropogon scoparius*, 7.75 mi. northwest of Thompson, Aug. 6, 1928. Not numerous.

*Prionosoma podopioides* Uhl.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Thyanta custator* Fabr.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Banasa dimidiata* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, one specimen.

*Stiretrus fimbriatus* Say

At *Spartina consocias*, 1 mi. south of Amana, Aug. 13, 1927, one specimen.

*Apateticus* sp.

At *Carex socias*, 7.75 mi. northwest of Thompson, June 30, 1928, two nymphs. One nymph was feeding on a small ground beetle when taken.

*Podisus maculiventris* Say

At *Spartina consocias*, and *Polygonum amphibium* socias, nearly all of the specimens, where they are common. Earliest adult, June 26, 1926; latest adult, Aug. 13, 1927.

*Merocoris distinctus* Dall.

At *Stipa spartea*—*Bouteloua curtipendula* association, 2 mi. north of Ames, July 11, 1928, four specimens. At *Andropogon furcatus* consocias, 2.5 mi. south of Ames, July 20, 1925, one specimen.

*Euthochtha galeator* Fabr.

Swept from *Solidago* sp., 1 mi. south of Amana, June 23, 1928, one specimen.

*Protenor belfragei* Hagl.

Taken on *Spartina consocias*, Lake Amana, Aug. 12, 31, 1927, seven specimens. At *Spartina consocias*, 6 mi. northwest of Cedar Falls, July 17, 1926, three specimens. At *Carex socias*, 7.75 mi. northwest of Thompson, Sept. 15, 1928, one specimen, and at *Stipa spartea*—*Andropogon scoparius* association, Aug. 6, 1928, two specimens.

*Alydus eurinus* Say

At *Andropogon furcatus* consocias, 2.5 mi. south of Ames, Aug. 7, 11, 1925. At *Spartina consocias*, 10 mi. southwest of Kelso, July 30, 1928, one specimen. On *Amorpha canescens*, 3 mi. south of Muscatine, Sept. 1, 1928. At *Cassia Chamaecrista*, in bloom, 1 mi. south of Amana, Aug. 25, 1928, several specimens. At *Andropogon furcatus* consocias, 6 mi. northwest of Cedar Falls, July 17, 1928, and at *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1927. Not numerous.

*Alydus conspersus* Montd.

At *Andropogon furcatus* consocieties, *Andropogon furcatus*—*Sorghastrum nutans* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, July 17, 1926; latest adult, Sept. 1, 1928. Breeding on *Amorpha canescens* at several stations. Common.

*Alydus pilosulus* H. S.

At *Andropogon furcatus* consocieties, *Andropogon furcatus*—*Sorghastrum nutans* associates, *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, July 17, 1926; latest adult, Oct. 20, 1928. On *Amorpha canescens*, 3 mi. south of Muscatine, Sept. 1, 1928. Not numerous.

*Harmostes reflexulus* Say

At *Andropogon furcatus* associates, *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, July 16, 1925; latest adult, Oct. 20, 1928. Not numerous.

*Ceraleptus americanus* Stal

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, one specimen.

*Aufeius impressicollis* Stal

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Corizus viridicatus* Uhl.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 25-Aug. 1, 1928, scarce. At *Bouteloua* community, Ocheyedan Mound, July 23, 1928, one specimen. At flower of *Dyssodia papposa*, Oak Grove State Park, July 25, 1928, one specimen.

*Corizus bohemanii* Sign.

Nearly all specimens are from *Spartina* consocieties, and *Polygonum amphibium* societies. Earliest adult, July 19, 1926; latest adult, Sept. 1, 1928. Common.

*Jalysus spinosus* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, July 26, Aug. 2, 1926, two specimens. On flowers of *Gaura parviflora*, 1 mi. south of Amana, Aug. 26, 1928. At *Andropogon furcatus* consocieties, 6 mi. northwest of Cedar Falls, July 17, 1928. Not numerous.

*Lygaeus kalmii* Stal

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, Aug. 17, 1925, June 19, 1926. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, Oak Grove State Park, July 25, 1928. Five specimens, in total, collected.

*Lygaeus bicrucis* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associes, nearly all specimens. Earliest adult, May 12, 1927; latest adult, Aug. 25, 1928. At flowers of *Cicuta maculata*, most frequently, where they are numerous at times.

*Ortholomus scolopax* Say

At *Andropogon furcatus* associes. At *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Stipa spartea*—*Andropogon scoparius* association, more frequent. Earliest adult, July 25, 1928; latest adult, Sept. 19, 1928. Not numerous.

*Nysius californicus* Stal

At *Andropogon furcatus* consocies, nearly all specimens, less at *Spartina* consocies. Earliest adult, July 17, 1926; latest adult, Aug. 26, 1926. At flower of *Silphium laciniatum*, July 15, 1926, and flowers of *Cicuta maculata*, Aug. 4, 1928. Not numerous.

*Nysius ericae* Schill.

At all communities higher than *Spartina* consocies. Earliest adult, April 17, 1927; latest adult, Aug. 5, 1928. Taken in largest numbers at *Bouteloua hirsuta*—*B. curtipendula* association, Ocheyedon Mound, July 23, 1928.

*Cymus angustatus* Stal

At *Spartina* consocies, 2.5 mi. south of Ames, May 12, 30, 1927, three specimens. At *Carex* socies, 1 mi. south of Amasa, June 23, 1928, one specimen.

*Cymus luridus* Stal

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, May 12, 1927, one specimen, and at *Spartina* consocies, 4 mi. northwest of Thompson, May 18, 1928, one specimen.

*Ischnodemus falicus* Say

At *Spartina* consocies, numerous. Earliest adult, April 16, 1927; latest adult, Aug. 25, 1928. Nymphs found only on *Spartina Michauxiana*; most frequently in July and August.

*Blissus leucopterus* Say

At *Polygonum amphibium* socies, 2 mi. west of Pacific Junction, July 31, 1928, one specimen.

*Geocoris uliginosus limbatus* Stal

At all communities higher than *Spartina* consocies. Earliest adult, June 16, 1926; latest adult, Aug. 14, 1926. Most frequently taken on ground; not numerous where sod is close, but easily obtained where grasses are more bunchy. A nymph obtained at *Bouteloua hirsuta*—*B. curtipendula* association, 2 mi. north of Ames, Aug. 18, 1926, moulted into an adult at the end of five days after feeding on four small leafhoppers.

*Phlegyas abbreviatus* Uhl.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, July 20, 1928, one specimen, and 6 mi. south of Washington, 2 specimens, Sept. 7, 1927. At *Spartina* consociates, 1 mi. south of Amana, Aug. 12, 1927, one specimen.

*Oedancala dorsalis* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 1 mi. south of Amana, Aug. 12, 13, 1927, June 23, Aug. 25, 1928, eight specimens of which five are from *Spartina* consociates. At *Spartina* consociates, 10 mi. southwest of Kelso, July 30, 1928, one specimen.

*Sphaerobius insignis* Uhl.

At all communities higher than *Spartina* consociates except *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, June 5, 1928; latest adult, Aug. 7, 1928. Most frequent at *Stipa spartea*—*Andropogon scoparius* association, Ocheyedan Mound, July 23, 1928, where they were one of the most common insects.

*Ligyrocoris diffusus* Uhl.

At all communities. Earliest adult, June 23, 1928; latest adult, Oct. 20, 1928. Most numerous at *Andropogon furcatus* associates, where herbaceous plant flowers attracted many of this species.

*Zeridoneus costalis* V. D.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, Aug. 6, 1928, and 6 mi. northwest of Ledyard, Aug. 7, 1928, four specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, one specimen.

*Pseudocnemodus canadensis* Prov.

At all communities higher than *Spartina* consociates except *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, June 28, 1928; latest adult, Oct. 20, 1928. Not numerous at any community.

*Emblethis vicarius* Horv.

At *Stipa spartea*—*Andropogon scoparius* association 2 mi. north of Ames, June 24, 1926, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, two specimens.

*Piesma cinerea* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, May 30, June 5, 1927, 8 mi. southeast of Britt. One specimen on each date.

*Acalypta lillianis* Bueno

Among moss of *Spartina* consociates, 2.5 mi. south of Ames, April 22, 1928, one nymph. In company with Dr. Drake, the author found seven nymphs in the same situation, Aug. 14, 1927. These nymphs lived until November, 1927, on moss indoors. Several nymphs were found on moss in *Andropogon furcatus* consociates, Oct. 9, 1928, 2.5 mi. south of Ames. Drake



(1928) has summarized the observations of several workers which connect the species with moss.

*Melanorhopala clavata* Stal

At *Andropogon furcatus* consocieties and *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, June 5, 1926; latest adult, July 28, 1925. At 1 mi. south of Amana two adult specimens were observed with beaks inserted into upper portion of a stem of *Solidago* sp., Aug. 13, 1928. One of the specimens appeared to feed for 15 minutes. These individuals were observed feeding on the same plant in a cage during several weeks in August. No evidence of the species breeding on this species of plant was obtained.

*Atheas mimeticus* Heid.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 17, 1926, one specimen. Numerous at a family of *Petalostemum candidum*, 7.75 mi. northwest of Thompson, Aug. 6, 1928, where nymphs and adults were found.

*Phymata erosa fasciata* Gray

At all communities, on flowers of various herbaceous plants. Earliest adult, July 10, 1925; latest adult, Oct. 20, 1928. Most numerous at *Andropogon* communities, where *Compositae* are frequent.

*Fitchia aptera* Stal

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 29, 1925, April 16, 1927, Mar. 25, 1928, one specimen at each date. Swept from *Anemone canadense*, 1 mi. south of Amana, June 23, 1928, one specimen.

*Sinea diadema* Fab.

At all *Andropogon* communities. Earliest adult, July 23, 1928; latest adult, Oct. 20, 1926. Common at several communities. Most of the specimens were at *Stipa spartea*—*Andropogon scoparius* association.

*Pagasa fusca* Stein

Swept from *Solidago canadensis*, 1 mi. south of Amana, Aug. 25, 1928, one specimen.

*Nabis subcoleoptratus* Kirby

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest collection, June 15, 1928; latest, Aug. 6, 1928. Numerous at *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, June 15, 1928, but not common at any other station. One of the specimens has long wings.

*Nabis propinquus* Reut.

At *Stipa spartea*—*Andropogon scoparius* association, 8 mi. southeast of Britt, July 6, 1928, two specimens.

*Nabis capsiformis* Germ.

At *Spartina* consocieties, 2 mi. west of Kelso, July 30, 1928, one specimen.

*Nabis ferus* Linn.

At all communities. Earliest adult, March 25, 1928; latest adult, Oct. 20, 1928. More numerous at communities higher than *Spartina* consocieties. At Ocheyedan Mound, July 23, 1928, adult specimen taken while feeding on a Hemipterous nymph.

*Nabis ferus* var. *pallidipennis* Harris

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, May 2, 1928; latest adult, Aug. 9, 1928. Most of the specimens are from *Spartina* consocieties, where it is probably one of the more common insects.

*Nabis alternatus* Parsh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 8 mi. southeast of Britt, Aug. 9, 1928, 2.5 mi. south of Ames, April 22, 1928. One specimen on each date. At *Andropogon scoparius*, 7.75 mi. northwest of Thompson, Aug. 6, 1928, one specimen.

*Xylocoris sordidus* Reuter

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 25, 1925, one specimen.

*Triphleps insidiosus* Say

At nearly all communities. Common at flowers, especially of *Compositae*. Earliest adult, May 19, 1926; latest adult, Sept. 15, 1928.

*Collaria oculata* Reut.

At *Spartina* consocieties, 1 mi. south of Amana, Aug. 12, 1927, one specimen.

*Miris dolabratus* Linn.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, where timothy (*Phleum pratense*) had invaded, 1 mi. south of Amana, June 23, 1928, six specimens.

*Stenodema vicinum* Prov.

At *Spartina* consocieties, 2.5 mi. south of Ames, July 15, 1926, and 7.75 mi. northwest of Thompson, Aug. 6, 1928. At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, Aug. 7, 1928. One specimen on each date.

*Trigonotylus ruficornis* Geoff.

At *Stipa spartea*—*Andropogon scoparius* association, 3.5 mi. north of Ledyard, Aug. 7, 1928, one specimen.

*Trigonotylus tarsalis* Reut.

At *Spartina* consocieties. Earliest adult, May 20, 1927; latest adult, Sept. 15, 1928. Numerous. Nymphs on *Spartina Michauxiana* numerous during August.

*Teratocoris discolor* Uhl.

At *Spartina* consocieties, 8 mi. southeast of Britt, Aug. 9, 1928, two specimens, and .5 mi. south of Missouri Valley, Aug. 1, 1928, six specimens.

*Teratocoris paludum* J. Sahlb.

At *Carex* societies, Lake Amana, June 23, 1928, one specimen.

*Platytyrellus nigricollis* Reut.

At *Andropogon furcatus* consocieties, 6 mi. northwest of Cedar Falls, July 17, 1926, two specimens.

*Mimoceps insignis* Uhl.

At *Spartina* consocieties and *Carex* societies, 1 mi. south of Amana, June 23, Aug. 12, 1928, three specimens. At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, Aug. 7, 1928.

*Neurocolpus nubilus* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, three specimens.

*Adelphocoris rapidus* Say

At all communities. Earliest adult, June 15, 1928; latest adult, Oct. 20, 1928. Most numerous at flowers of *Compositae* of *Andropogon furcatus* consocieties, but common at all species of blooming herbaceous plants.

*Polymerus basalis* Reut.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 27, 1925, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. north of Reels City, Aug. 1, 1928, six specimens.

*Polymerus basalis fuscatus* Knight

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Sept. 24, 1926, one specimen.

*Polymerus chrysopsis* Knight

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, two specimens.

*Polymerus venaticus* Uhl.

At *Carex* societies, Lake Amana, June 23, 1928, and swept from *Solidago canadensis*, 1 mi. south of Amana, June 23, 1928. One specimen at each location.

*Capsus simulans* Stal

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 21, 1926, one specimen.

*Lygus pratensis* Linn.

At all communities. Earliest adult, Mar. 21, 1926; latest adult, Sept. 19, 1928. Most numerous at higher associations such as *Bouteloua hirsuta*—*B. curtipendula*, *Andropogon scoparius*—*Bouteloua curtipendula*, and *Stipa spartea*—*Andropogon scoparius*. One of the common insects of these associations at flowers of herbaceous plants.

*Lygus pratensis oblineatus* Say

At all communities. Most numerous at *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Mar. 23, 1926; latest adult, Oct. 20, 1928. Adults and nymphs numerous at flowers of *Erigeron ramosus*, 1 mi. south of Amana, June 23, 1928. Adults numerous at flowers on *Anemone canadense*, same location and date. Numerous at flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928. One of the most numerous insects of the lower communities.

*Lygus pratensis strigulatus* Walk.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 31, 1926, one specimen. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, 1928, one specimen.

*Lygus plagiatus* Uhl.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, chiefly. Earliest adult, June 23, 1928; latest adult, Sept. 19, 1928. Twelve of the fifteen specimens were taken at *Spartina* consocieties. Not numerous at any station.

*Lygus campestris* Linn.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Mar. 21, 1928; latest adult, Aug. 9, 1928. Taken most frequently at flowers of *Cicuta maculata*. A common insect in *Spartina* consocieties where *Cicuta maculata* is abundant.

*Deraeocoris histrio* Reut.

At *Polygonum amphibium* societies. Earliest adult, May 12, 1927; latest adult, Sept. 16, 1928. Sometimes numerous. A nymph, taken Aug. 4, 1928, fed upon a small Lepidopterous larva taken on *Polygonum amphibium* and a leafhopper (*Dikraneura fieberi*). It fed upon leaf of *Polygonum amphibium*, Aug. 7, 1928. Molted into adult Aug. 7, 1928.

*Coquillettia mimetica* Osborn

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. northeast of Beloit, July 25, 1928, common. A few nymphs were taken from *Bouteloua curtipendula*, and the adults seemed more numerous at the same grass.

*Dicyphus vestitus* Uhl.

At *Spartina* consocieties, 2 mi. west of Kelso, July 30, 1928, one specimen.

*Halticus intermedius* Uhl.

At *Andropogon scoparius*—*Bouteloua curtipendula* association 15 mi. north of Sioux City, one specimen, July 26, 1928.

*Strongylocoris* sp.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, June 23, 1928; latest adult, Aug. 12, 1928. Most of the specimens were taken 1 mi. south of Amana. The species was most readily obtained at *Solidago canadensis* at the several stations.



*Ceratopsus modestus* Uhl.

At *Andropogon furcatus* consocias, 2.5 mi. south of Ames, Aug. 4, 1927, one specimen.

*Lopidea media* Say

Taken on *Solidago* sp., 7.75 mi. northwest of Thompson, June 30, 1928, one specimen, and on *Solidago canadensis*, 1 mi. south of Amana, June 23, 1928, one specimen. Others seen.

*Lopidea minor* Knight

The author (1928) took this specimen at a *Stipa*—*Bouteloua* community, and found it showed a preference for *Pelatostemum purpureum*. Not taken elsewhere.

*Lopidea teton* Knight

At *Stipa spartea*—*Andropogon scoparius* association, where its food plant, *Astragalus caryocarpus*, was present, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen. More seen.

*Lopidea incurva* Knight

Appeared to be feeding on flower bud of *Lepachys pinnata*, 1 mi. south of Amana, July 20, 1928. One specimen.

*Psallus* sp.

Swept from *Aster sericeus*, northeast of Iowa State College grounds, July 11, 1928. One of the specimens was observed to feed on *A. sericeus*. Numerous at this location. Two specimens, swept from *Amorpha canescens*, 1.5 mi. northeast of Ocheyedan, July 23, 1928. One specimen at *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928.

*Plagiognathus politus* Uhl.

At all communities lower than *Bouteloua hirsuta*—*B. curtipendula*. Earliest adult June 23, 1928; latest adult Sept. 19, 1928. Most numerous at *Andropogon furcatus*—*Sorghastrum nutans* associates. Swept from flowers of *Anemone canadensis*, *Senecio aureus*, *Erigeron ramosus*, *Onosmodium occidentale*, *Rudbeckia hirta*, *Solidago* sp., and *Eupatorium* sp.

*Plagiognathus davisi* Knight

The author (1928) reported one specimen from a *Stipa*—*Bouteloua* community, but has not taken it elsewhere.

*Chlamydatus associatus* Uhl.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, two specimens. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, July 7, 1928, and Ocheyedan Mound, July 23, 1928. One specimen at each date. At *Polygonum amphibium* socias, 2 mi. west of Pacific Junction, July 31, 1928, one specimen.

*Ilnacora divisa* Reut.

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, July 20, 1928, 1 mi. south of Amana, on *Helianthus grosseserratus*; latest adult, Aug. 24, 1928, 6 mi. south of Washington, swept from *Solidago Riddellii*. Taken at several stations on *Helianthus* sp., such as *occidentale* and *Maximiliani*. More readily obtained in western part of Iowa, where sunflowers are more generally distributed than elsewhere.

*Ilnacora stalii* Reut.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, July 14, 1926; latest adult, Aug. 24, 1928. Nearly all the specimens were swept from *Helianthus grosseserratus* and *H. Maximiliani*. Nymphs were seen on *H. grosseserratus*, 10 mi. southwest of Ames, July 14, 1928, where the species was numerous.

*Ilnacora* sp.

At *Andropogon scoparius*—*Bouteloua curtipendula* association. Nearly all the specimens were swept from *Dyssodia papposa*, July 24-31, 1928. Nymphs were seen on *D. papposa*, Gitchie-Manito State Park, July 24, 1928.

*Labopidea planifrons* Knight

At *Bouteloua hirsuta*—*B. curtipendula* association, 1.5 mi. northeast of Ochevedan, July 23, 1928, one specimen.

*Reuteroscopus ornatus* Reut.

Swept from blooming *Cassia Chamacrista*, 1 mi. south of Amana, Aug. 25, 1928, one specimen.

*Saldula major* Prov.

At *Polygonum amphibium* sociies, .5 mi. south of Missouri Valley, Aug. 1, 1928, one specimen.

*Saldula interstitialis* Say

At *Spartina* consociies, 2.5 mi. south of Ames, May 30, 1927, Mar. 25, April 22, 1928. Four specimens.

*Micracanthia humeralis* Say

At *Andropogon furcatus* consociies, 2.5 mi. south of Ames, May 12, 1927. At *Spartina* consociies, May 30, June 5, 1927. Four specimens.

## ORDER HOMOPTERA

The determinations of species were made by Drs. E. D. Ball, D. W. DeLong, P. B. Lawson, F. C. Hottes, and Messrs. Wm. T. Davis and G. S. Walley. The arrangement follows Van Duzee (1917), mainly.

*Okanagana balli* Davis

At stands of *Andropogon furcatus*, chiefly. Earliest adult, June 26, 1926; latest adult, July 7, 1928. More specimens were obtained in 1928 than in the three previous years. Not numerous in 1928.

*Melampsalta calliope* Walk.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 6, 1928, two specimens. At *Andropogon furcatus*—*Sorghastrum nutans* associes, 1 mi. south of Amana, July 20, 1928, one specimen.

*Lepyronia quadrangularis* Say

At *Polygonum amphibium* socies, 10 mi. southwest of Kelso, July 30, 1928, one specimen. At *Andropogon furcatus*—*Sorghastrum nutans* as-socies, Lacey-Keosauqua State Park, Aug. 22, 1928, one specimen. At *Koeleria cristata*, 1.5 mi. northeast of Ocheyedon, July 23, 1928, one spec-imen.

*Lepyronia gibbosa* Ball

At *Andropogon furcatus* consocies, 8 mi. southeast of Britt, July 6, 1928; *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, and Council Bluffs, July 31, 1928; *Stipa spartea*—*Andropogon scoparius* association, 1.5 mi. northeast of Ocheyedon, July 23, 1928, and 7 mi. northwest of Thompson, June 30, 1928. Four of the five specimens are from the two higher communities.

*Philaronia bilineata* Say

At *Bouteloua hirsuta*—*B. curtipendula* association, closely pastured, Ocheyedon Mound, July 23, 1928, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, four specimens.

*Ceresa diceros* Say

At *Spartina* consocies, 1 mi. south of Amana, Aug. 12, 1927, one speci-men. Several seen, and one specimen collected from *Helianthus grosseserratus*, 1 mi. south of Amana, July 20, 1928. At *Andropogon furcatus* con-socies, 6 mi. northwest of Cedar Falls, July 17, 1926, one specimen.

*Ceresa bubalus* Fab.

At *Andropogon furcatus*—*Spartina Michauxiana* associes. Earliest adult, July 16, 1928; latest adult, Aug. 25, 1928. Taken on *Solidago cana-densis*, *Helianthus grosseserratus*, and most frequently at *Polygonum am-phibium* socies, where it was, at times, common.

*Ceresa constans* Walk.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Ser-geant Bluff, July 26, 1928, one specimen.

*Stictocephala inermis* Fabr.

At *Andropogon furcatus* communities, *Spartina* consocies, *Stipa spar-tea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bou-teloua curtipendula* association. Earliest adult, June 23, 1928; latest adult, Aug. 7, 1928. One nymph taken from *Solidago canadensis*, 1 mi. south of Amana, June 23, 1928. Adults swept from *Amorpha canescens*, *Helianthus* sp. and *Anemone canadensis*. Common at several communities.

*Stictocephala lutea* Walk.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 17, 1926, three specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, May 18, June 30, 1928, two specimens.

*Acutalis tartarea* Say

At *Spartina* consocieties, 6 mi. northwest of Cedar Falls, July 17, 1926, three specimens.

*Acutalis semicrema* Say

On *Helianthus grosseserratus*, 7.75 mi. northwest of Thompson, Aug. 6, 1928, five specimens.

*Micrutalis calva* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 9, 1927, two specimens. On *Solidago* sp., 1 mi. south of Amana, June 23, Aug. 25, 1928, two specimens.

*Vanduzeeia triguttata* Burm.

At *Stipa spartea*—*Andropogon scoparius*, and *Andropogon scoparius*—*Bouteloua curtipendula* associations. Earliest adults, June 30, 1928; latest adult, Aug. 19, 1928. Taken on *Amorpha canescens* at several stations. Common where *A. canescens* occurred in good stands.

*Publilia concava* Say

On *Solidago canadensis*, *S. Riddellii*, and *Helianthus grosseserratus*, Aug. 24, 1928, Sept. 7, 1927. Not numerous.

*Publilia modesta* Uhl.

At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Reared from nymph on *Helianthus grosseserratus*; specimen labelled, 5 mi. south of Stanhope, Aug. 19, 1927. Moulded twice in twelve days. Taken from stems of *Solidago rigida*, 7.75 mi. northwest of Thompson, Aug. 6, 1928. Not numerous.

*Campylenchia latipes* Say

At all communities above *Spartina* consocieties. Earliest adult, July 10, 1925; latest adult, Sept. 19, 1928. Common, and most numerous at *Stipa spartea*—*Andropogon scoparius* association.

*Enchenopa binotata* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, swept from *Ceanothus americanus*, 4 mi. northeast of Beloit, July 25, 1928, three specimens.

*Agallia 4-punctata* Prov.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 23, 1926, one specimen.



*Agallia sanguinolenta* Prov.

At all communities. Earliest adult, 2.5 mi. south of Ames, Mar. 25, 1928; latest adult, Sept. 15, 1928. Numerous at *Stipa spartea*—*Andropogon scoparius* association, and less at other communities.

*Agallia uhleri* Van D.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, Aug. 26, 1926, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 2.5 mi. north of Ames, Oct. 1, 1926, 7.75 mi. northwest of Thompson, two specimens.

*Agallia cinerea* O. & B.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 25-30, 1928, scarce.

*Idiocerus alternatus* Fitch

At *Andropogon furcatus*—*Sorghastrum nutans* associes, 1.5 mi. north-east of Muscatine, Sept. 1, 1928, one specimen.

*Oncometopia lateralis* Fabr.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 30, 31, 1928. Common at Hamburg State Park, July 30, 1928.

*Cicadella hieroglyphica* var. *dolobrata* Ball

On *Helianthus Maximiliani*, 10 mi. southwest of Kelso, several seen, one specimen taken July 30, 1928. At *Polygonum amphibium* sociés, 2 mi. west of Pacific Junction, July 31, 1928, one specimen.

*Cicadella gothica* Sign.

Swept from *Solidago canadensis*, 6 mi. south of Washington, Aug. 24, 1928, one specimen.

*Helochara communis* Fitch

At *Spartina* consociés, 2.5 mi. south of Ames, May 12, 1927, one specimen.

*Graphocephala coccinea* Forst.

Swept from *Solidago canadensis*, 1 mi. south of Amana, at edge of wooded area, Aug. 25, 1928, four specimens.

*Draeculacephala mollipes* Say

At all communities. Earliest adult, June 15, 1928; latest adult, Sept. 19, 1928. Common at *Andropogon furcatus* consociés, *Andropogon furcatus*—*Sorghastrum nutans* associes, and *Stipa spartea*—*Andropogon scoparius* association. Scarce, usually at other communities.

*Draeculacephala noveboracensis* Fitch

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, June 23, 1928; latest adult, Sept. 15, 1928. Most numerous at *Spartina* consociés.

*Gypona octolineata* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, July 26, 1928; latest adult, Aug. 7, 1928. Not numerous.

*Gypona octolineata* var. *striata* Burm.

At nearly all communities, but scarce. Earliest adult, July 23, 1928; latest adult, Aug. 12, 1927.

*Gypona melanota* Spangb.

At *Andropogon furcatus*—*Spartina Michauxiana* associes, *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Most frequently taken at communities dominated by *Andropogon furcatus*. Earliest adult, July 10, 1925; latest adult, Aug. 22, 1925. Not numerous.

*Gypona unicolor* Stal

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, one specimen. At *Spartina* consocies, 7.75 mi. northwest of Thompson, Aug. 6, 1928, one specimen.

*Prairiana cinerea* Uhl.

At *Andropogon furcatus* consocies, 6 mi. northwest of Cedar Falls, July 17, 1926, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 3.5 mi. north of Ledyard, July 7, 1928, and 6 mi. northwest of Ledyard, Aug. 7, 1928, two specimens.

*Xerophloea viridis* Fabr.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 26-31, 1928. Not numerous.

*Stroggylocephalus agrestis* Fall.

At *Spartina* consocies, 2.5 mi. north of Ames, May 7, 1928, one specimen.

*Xestocephalus pulicarius* Van D.

At *Stipa spartea*—*Andropogon scoparius* association, mown, 7.75 mi. northwest of Thompson, Sept. 15, 1928, one specimen.

*Dorycephalus platyrhynchus* Osborn

At *Andropogon furcatus*—*Spartina Michauxiana* associes, and *Stipa spartea*—*Andropogon scoparius* association. Adults, May 30-June 30. Nymphs, May 9-18; Aug. 6-Sept. 15. Not numerous, most of specimens from *Andropogon furcatus* consocies.

*Hecalus lineatus* Uhl.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, July 10, 1925, one specimen. At *Spartina* consocies, 1 mi. south of Amana, Aug. 25, 1928, 7.75 mi. northwest of Thompson, Aug. 6, 1928, three specimens.

*Parabolocratus viridis* Uhl.

At all communities. Earliest adult, May 19, 1928; latest adult, Aug. 9, 1928. Common at *Stipa spartea*—*Andropogon scoparius* association.

*Parabolocratus major* Osborn

At *Spartina* consocieties, 2.5 mi. south of Ames, June 26, 1926, Lake Amana, Aug. 12, 1927, two specimens. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 14, 1925, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, Aug. 6, 1928, one specimen.

*Parabolocratus flavidus* Sign.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 30, 1927, one male specimen.

*Mesamia nigradorsum* Ball

Taken on *Helianthus grosseserratus*, 2.5 mi. south of Ames, Aug. 14, 1925, and 10 mi. southwest of Ames, July 14, 1928. Two specimens at each date, and others seen on the sunflower plants.

*Scaphoideus immistus* Say

At *Spartina* consocieties, 2.5 mi. north of Ames, Aug. 5, 1927, one specimen.

*Platymetopius acutus* Say

At all communities lower than *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, June 23, 1928; latest adult, Sept. 19, 1928. Scarce at all communities.

*Platymetopius cinereus* O. & B.

At *Andropogon furcatus* consocieties, *Andropogon furcatus*—*Sorghastrum nutans* associates, *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, June 23, 1928; latest adult, Sept. 16, 1928. Not numerous at any community.

*Platymetopius frontalis* Van D.

At *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, June 23, 1928; latest adult, Sept. 5, 1928. Nearly all specimens were swept from *Solidago canadensis*, at which it was common.

*Deltocephalus delector* S. & DeL.

At *Spartina* consocieties, 1 mi. south of Amana, June 23, 1928, one specimen.

*Deltocephalus areolatus* Ball

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Deltocephalus albidus* O. & B.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. northeast of Beloit, July 25, 1928, one specimen, and at *Stipa spartea*—

*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, Aug. 6, 1928, one specimen.

*Deltocephalus sandersi* Osborn

At *Andropogon furcatus* consociates, 2.5 mi. south of Ames, July 16, Aug. 11, 1925, four specimens.

*Deltocephalus visendus* Crumb

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, Aug. 6, 1928, and 6 mi. northwest of Ledyard, Aug. 7, 1928. One specimen at each location.

*Deltocephalus reflexus* O. & B.

At *Andropogon furcatus* consociates, 2.5 mi. south of Ames, July 10, Aug. 11, 25, 1925, three specimens. At *Stipa spartea*—*Bouteloua curtipendula* association, 2 mi. north of Ames, Oct. 9, 1926, one specimen.

*Deltocephalus pectinatus* O. & B.

At *Andropogon furcatus* consociates, 5 mi. northwest of Buffalo Center, Sept. 16, 1928, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 3.5 mi. north of Ledyard, Aug. 7, 1928, one specimen.

*Deltocephalus abbreviatus* O. & B.

At *Bouteloua hirsuta*—*B. curtipendula* association, Ocheyedon Mound, July 23, 1928, two specimens.

*Deltocephalus stylatus* Ball

At *Bouteloua hirsuta*—*B. curtipendula* association, Ocheyedon Mound, and 1.5 mi. northeast of Ocheyedon, July 23, 1928, five specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. south of Westfield, July 26, 1928, one specimen.

*Deltocephalus configuratus* Uhl.

At all communities higher than *Spartina* consociates. Earliest adult, May 18, 1928; latest adult, Aug. 9, 1928. Numerous at *Stipa spartea*—*Andropogon scoparius* association. Less taken at other communities.

*Deltocephalus sayi* Fitch

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, Aug. 13, 1928. Not numerous.

*Deltocephalus inimicus* Say

At all communities lower than *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, June 15, 1928; latest adult, Sept. 15, 1928. Numerous at *Andropogon furcatus* consociates, *Andropogon furcatus*—*Sorghastrum nutans* associates, and *Stipa spartea*—*Andropogon scoparius* association.



*Deltocephalus collinus* Boh.

At *Stipa spartea*—*Andropogon scoparius* association, 3.5 mi. north of Ledyard, Aug. 7, 1928, 6 mi. northwest of Ledyard, Aug. 6, 1928, and 7.75 mi. northwest of Thompson, Aug. 6, 1928. Scarce.

*Deltocephalus striatus* Linn.

At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, Aug. 9, 1928, three specimens. At *Spartina* consocieties, 6 mi. northwest of Ledyard, July 7, 1928, and .5 mi. south of Missouri Valley, Aug. 1, 1928. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 10, 1927, Oct. 20, 1928. One to three specimens at each of the above mentioned communities.

*Deltocephalus unicoloratus* G. & B.

At *Andropogon furcatus* consocieties, *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, July 7, 1928; latest adult, Sept. 15, 1928. Not numerous.

*Deltocephalus paludosus* Ball

At *Carex* societies, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Athysanella magdalena* Bak.

At *Stipa spartea*—*Andropogon scoparius* association, 6 mi. northwest of Ledyard, Aug. 7, 1928, five specimens. At *Bouteloua hirsuta*—*B. curtipendula* association, closely grazed, July 23, 1928, Ocheyedon Mound, one specimen.

*Driotura gammaroides* Van D.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, Aug. 6, 1928. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, and Sergeant Bluff, July 26, 1928. At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, July 7, 1928, and 5 mi. east of Renwick, Aug. 9, 1928. One specimen at each date.

*Driotura gammaroides* var. *flava* O. & B.

At *Andropogon furcatus* consocieties, 5 mi. east of Renwick, Aug. 9, 1928, 2.5 mi. south of Ames, Aug. 4, 1927. One specimen at each location.

*Driotura robusta* O. & B.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. south of Westfield, Oak Grove State Park, Sergeant Bluff, July 25-26, 1928, four specimens. At *Stipa spartea*—*Andropogon scoparius* association, 1.5 mi. northeast of Ocheyedon, July 23, 1928, one specimen.

*Euscelis magnus* O. & B.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, June 9, 1926, July 4, 1928, two specimens.

*Euscelis exitiosus* Uhl.

At *Andropogon furcatus* consocies, *Stipa spartea*—*Bouteloua curtipendula* association, and *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, July 7, 1928; latest adult, Sept. 24, 1926. Not numerous.

*Euscelis striolus* Fall.

At *Andropogon furcatus* consocies, 8 mi. southeast of Britt, July 6, 1928, one specimen.

*Euscelis paralellus* Van D.

At *Stipa spartea*—*Andropogon scoparius* association, 6 mi. northwest of Ledyard, July 7, 1928, one specimen.

*Euscelis extrusus* Van D.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, two specimens.

*Euscelis obsoletus* Kirsch

At *Carex* sociies, Lake Amana, June 23, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Euscelis anthracinus* Van D.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, Aug. 17, 1926, April 24, 1927, three specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, and Sergeant Bluff, July 26, 1928, four specimens.

*Euscelis striatulus* Fall.

At *Stipa spartea*—*Andropogon scoparius*, and *Andropogon scoparius*—*Bouteloua curtipendula* association, nearly all of the specimens. Earliest adult, July 23, 1928; latest adult, Aug. 19, 1927. Numerous.

*Euscelis comma* Van D.

At all communities except *Andropogon scoparius*—*Bouteloua curtipendula*, and *Bouteloua hirsuta*—*B. curtipendula* associations. Earliest adult, June 15, 1928; latest adult, Aug. 13, 1927. Not numerous.

*Euscelis colon* O. & B.

At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, June 30, 1928; latest adult, Aug. 9, 1928. Scarce.

*Euscelis curtisii* Fitch

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, July 10-Aug. 11, 1925, Aug. 26, 1926. At *Carex* sociies, Lake Amana, June 23, 1928, one specimen. Not numerous at either station.

*Euscelis obtutus* Van D.

At *Andropogon furcatus* consocies, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, April 16, 1927; latest adult, Sept. 17, 1926. Not numerous.

*Euscelis obscurinervis* Stal

At all communities above *Spartina* consocieties except *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, July 30, 1928; latest adult, Sept. 15, 1928. Scarce.

*Phlepsioides areolatus* Bak.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1927, one specimen.

*Phlepsioides altus* O. & B.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 24-30, 1928, common. At *Bouteloua hirsuta*—*B. curtipendula* association, Ocheyedon Mound, and 1.5 mi. northeast of Ocheyedon, July 23, 1928, numerous. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 5, 1927, and 7 mi. northwest of Thompson, Aug. 6, 1928, scarce.

*Phlepsioides irroratus* Say

At all communities except *Bouteloua hirsuta*—*B. curtipendula*, and *Andropogon scoparius*—*Bouteloua curtipendula* associations. More specimens from *Polygonum amphibium* societies, 2.5 mi. south of Ames, than from any other community. Earliest adult, June 19, 1926; latest adult, Sept. 15, 1928.

*Phlepsioides nebulosus* O. & B.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 4, 1925, one specimen.

*Phlepsioides solidaginis* Walk.

At *Spartina* consocieties, 2.5 mi. north of Ames, Aug. 5, 1927, two specimens.

*Dorydiella floridana* Bak.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 29, 31, 1925, two specimens.

*Thamnotettix melanogaster* Prov.

At *Carex* societies, chiefly. Earliest adult, June 26, 1926; latest adult, Sept. 15, 1928. Not numerous.

*Thamnotettix ciliatus* Osborn

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1927, one specimen.

*Thamnotettix decipiens* Prov.

At *Polygonum amphibium* societies, 3.5 mi. north of Ledyard, July 7, 1928, one specimen.

*Thamnotettix fitchii* Van D.

At *Spartina* consocieties, 1 mi. south of Amana, Aug. 13, 1927, one specimen.

*Chlorotettix unicolor* Fitch

At all communities higher than *Spartina* consocieties. Earliest adult, June 23, 1928; latest adult, Sept. 7, 1927. More at *Stipa spartea*—*Andropogon scoparius* association than at any other community. Not numerous.

*Chlorotettix spatulatus* O. & B.

At all communities higher than *Spartina* consocieties. Earliest adult, July 6, 1925; latest adult, Sept. 1, 1928. Most numerous at *Andropogon scoparius*—*Bouteloua curtipendula* association.

*Chlorotettix tergatus* Fh.

At *Spartina* consocieties, 1 mi. south of Ames, Aug. 13, 1927, one specimen.

*Jassus olitorius* Say

At *Spartina* consocieties, 1 mi. south of Amana, Aug. 13, 31, 1927; on *Solidago canadensis*, 1 mi. south of Amana, Aug. 25, 1928, two specimens. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1927, one specimen.

*Cicadula sexnotata* Fall.

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association, and *Andropogon scoparius*—*B. curtipendula* association. Earliest adult, May 9, 1926; latest adult, Sept. 15, 1928. Most numerous at *Andropogon furcatus* consocieties.

*Eugnathodus abdominalis* Van D.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. northeast of Beloit, July 25, 1928, and Sergeant Bluff, July 26, 1928. At *Stipa spartea*—*Andropogon scoparius* association, 6 mi. northwest of Ledyard, Aug. 7, 1928. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1.5 mi. east of Muscatine, Sept. 1, 1928. Scarce.

*Dikraneura fieberi* Loew

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 25, 1925. At *Polygonum amphibium* societies, 2.5 mi. south of Ames, June 26, 1926. At *Spartina* consocieties, 5 mi. east of Renwick, Aug. 9, 1928. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, Sept. 15, 1928. Scarce.

*Empoasca obtusa* Walsh

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, 1928, two specimens, and 6 mi. south of Washington, Sept. 7, 1928, one specimen.

*Empoasca flavescens* Fabr.

Swept from *Rudbeckia hirta*, 1 mi. south of Amana, June 23, 1928, one specimen.

*Empoasca fabae* Harr.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928. At *Stipa spartea*—*Andropogon scoparius*



association, 5 mi. south of Stanhope, June 15, 1928. On *Helianthus grosseserratus*, 7.75 mi. northwest of Thompson, Aug. 6, 1928. Scarce.

*Scolops sulcipes* Say

At all communities higher than *Spartina* consocieties. Most numerous at *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 23, 1928; latest adult, Sept. 19, 1928.

*Scolops osborni* Ball

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. northeast of Beloit, July 25, 1928, two specimens, and Gitchie-Manito State Park, July 24, 1928, two specimens.

*Scolops spureus* Uhl.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, two specimens.

*Scolops vanduzeei* Ball

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, one specimen.

*Scolops angustatus* Uhl.

At all communities higher than *Spartina* consocieties except *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, July 11, 1928; latest adult, Aug. 7, 1928. Most numerous at *Bouteloua hirsuta*—*B. curtipendula* association, at Ocheyedan Mound, and 1.5 mi. northeast of Ocheyedan, and at *Andropogon scoparius*—*Bouteloua curtipendula* association, several stations.

*Scolops pungens* Germ.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 24-Aug. 1, 1928, at stations from Council Bluffs to Gitchie-Manito Park. Common at several stations.

*Phylloscelis pallescens* Germ.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1927, one specimen.

*Oliarus complectus* Ball

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, July 15, 1926, and Aug. 4, 1927, two specimens.

*Oliarus humilis* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10-31, 1925, four specimens. At *Spartina* consocieties, 2.5 mi. south of Ames, June 26, 1928, one specimen.

*Cixius basalis* V. D.

At *Spartina* consocieties and *Carex* societies, 7.75 mi. northwest of Thompson, Sept. 15, 1928, two specimens.

*Cixius stigmatus* Say

At *Spartina* consocieties, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Bruchomorpha oculata* Newm.

At *Stipa spartea*—*Andropogon scoparius* association, northeast of Iowa State College grounds, July 11, 1928, one specimen, and 5 mi. south of Stanhope, Oct. 20, 1928, one specimen.

*Bruchomorpha dorsata* Fitch

At all communities except *Bouteloua hirsuta*—*B. curtipendula*. Earliest adult, June 23, 1926; latest adult, Sept. 15, 1928. Largest number taken at one station was from *Andropogon scoparius*—*Bouteloua curtipendula* association, July 26, 1928, 4 mi. south of Westfield. Not numerous at any station.

*Aphelonema histrionica* Stal

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 25, Aug. 17, 26, 1926, five specimens. At *Spartina* consocieties, 2.5 mi. south of Ames, July 11, 1928, one specimen.

*Aphelonema bivittata* Ball

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, one specimen.

*Aphelonema simplex* Uhl.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 17, 1926, two specimens. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, Aug. 25, 1928, five specimens

*Acanalonia bivittata* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, several stations, July 25-31, 1928. At *Spartina* consocieties, 10 mi. southwest of Kelso, July 30, 1928. At *Andropogon furcatus*—*Sorghastrum nutans* associates at earliest date, Aug. 12, 1928, and latest date, Sept. 7, 1927. Found at stations bordered by woods or shrubs most frequently, but not numerous.

*Ormenis pruinosa* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 26-Aug. 1, 1928. Not numerous. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1927, one specimen.

*Cedusa vulgaris* Fh.

At *Carex* societies, 7.75 mi. northwest of Thompson, Sept. 15, 1928, two specimens.

*Herpis obscura* Ball

At *Carex* societies, 10 mi. southwest of Kelso, July 30, 1928, 7.75 mi. northwest of Thompson, Sept. 15, 1928, three specimens. Swept from *Amorpha canescens*, 1 mi. south of Amana, July 20, 1928, two specimens.

*Stenocranus dorsalis* Fitch

At *Spartina* consocieties, 2.5 mi. south of Ames, April 26, 1926, two specimens, May 12, 1927, one specimen, and March 25, 1928, one specimen.

*Stenocranus vittatus* Stal

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 4, 1927, one specimen.

*Kelisia crocea* Van D.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 23, 1928; latest adult, Sept. 15, 1928. Not numerous at any station, but more were taken at *Spartina* consocieties than at any other community.

*Pissonotus delicatus* Van D.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 25-31, 1928. Most frequent on *Dyssodia papposa*, Oak Grove State Park, July 25, 1928.

*Stobaera tricarinata* Say

At *Stipa spartea*—*Bouteloua curtipendula* association, 2 mi. north of Ames, Oct. 1, 1926, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 5, 12, May 21, 1926; one specimen on each date. At *Bouteloua hirsuta*—*B. curtipendula* association, 5 mi. south of Stanhope, Oct. 20, 1928, two specimens.

*Liburnia* near *osborni* V. D.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, chiefly. Earliest adult, May 18, 1926; latest adult, Sept. 15, 1928. Most frequent at *Spartina* consocieties.

*Livia vernalis* Fitch

At *Spartina* consocieties, 2.5 mi. south of Ames, May 12, 1927, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 11, 1928, one specimen.

*Aphalara calthae* Linn.

At *Carex* sociies, chiefly. Earliest adult, May 18, 1928; latest adult, Sept. 15, 1928. Not numerous.

*Aphalara veaziei* Patch

On *Solidago canadensis*, *S. missouriensis*. Earliest adult, July 11, 1928; latest adult, Aug. 25, 1928, several stations.

*Anoecia corni* Fab.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Sept. 19, 1928.

*Bipersona torticauda* Gill.

On *Cirsium iowense*, 5 mi. northwest of Buffalo Center, Aug. 6, 1928.

*Hyalopterus arundinis* Fab.

On *Phragmites communis*, 5 mi. northwest of Buffalo Center, Aug. 6, 1928, one specimen.

*Tritogenaphis* (?) *rudbeckiae* Fitch

On *Solidago rigida*, 7 mi. northwest of Thompson, June 30, 1928; on *Lepachys pinnata*, 1 mi. south of Amana, July 20, 1928.

*Tritogenaphis* sp.

On *Helianthus grosseserratus*, 2.5 mi. south of Ames, Aug. 18, 1927.

## ORDER COLEOPTERA

Most of the species have been determined by Prof. H. F. Wickham, Drs. L. L. Buchanan and G. M. Stirrett, and Messrs. W. J. Brown, K. F. Chamberlain, N. K. Bigelow (assisted by Chas. W. Leng), Chas. Schaeffer, and M. C. Lane. A few determinations were made by the author in common species. The arrangement follows Leng (1920).

*Cicindela punctulata* Oliv.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 11, 1928, one specimen. At *Bouteloua hirsuta*—*B. curtipendula* association, Ocheyedon Mound, July 23, 1928, 5 mi. south of Stanhope, Aug. 19, 1927, Aug. 9, 1928, one specimen at each date, and several seen. On rocks, and over bare places around rocks, *Andropogon scoparius*—*B. curtipendula* association, Gitchie-Manito State Park, July 24, 1928, several specimens of this species, probably, were seen.

*Calosoma calidum* Fab.

At *Spartina* consocieties, under debris, 2.5 mi. north of Ames, Aug. 5, 1927, one specimen.

*Scarites subterraneus* var. *substriatus* Hald.

At *Spartina* consocieties, subterranean, 1 mi. south of Gruver, July 8, 1928, one specimen.

*Dyschirius globulosus* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, April 21, 1926, one specimen. At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, May 19, 1928, seven specimens.

*Bembidion variegatum* Say

At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen, and 8 mi. southeast of Britt, May 19, 1928, three specimens.

*Bembidion affine* Say

At *Spartina* consocieties, 2.5 mi. south of Ames, Mar. 25, 1928, one specimen, and at *Andropogon furcatus* consocieties, 5 mi. east of Renwick, May 19, 1928, one specimen.

*Bembidion frontale* Lec.

At *Spartina* consocieties, 2.5 mi. north of Ames, May 2, 1928, one specimen, and 8 mi. southeast of Britt, May 19, 1928, three specimens.



*Tachys incurva* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 8, 1925, one specimen. At *Spartina* consocieties, 8 mi. southeast of Britt, May 19, 1928, one specimen, and at *Andropogon furcatus* consocieties, same locality and date.

*Tachyura vivax* Lec.

At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, July 6, 1928, one specimen.

*Eumolops sodalis* Lec.

Under stones, *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Apr. 25, 1928, and 2 mi. north of Ames, May 7, 1928. Five specimens.

*Eumolops colossus* Lec.

At *Spartina* consocieties, under debris, and subterranean, 2.5 mi. south of Ames, July 18, 1926, 2.5 mi. north of Ames, July 6, 1928, and 1 mi. south of Gruver, July 8, 1928. Four specimens.

*Abacidus permundus* Say

At *Spartina* consocieties, under debris, 2.5 mi. north of Ames, May 2, 1928, one specimen.

*Poecilus chalcites* Say

At *Spartina* consocieties, 2.5 mi. north of Ames, May 2, 1928, one specimen, under debris.

*Poecilus lucublandus* Say

At *Spartina* consocieties, under debris, 2.5 mi. north of Ames, May 2, 1928, two specimens.

*Omaseus luctuosus* Dej.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Mar. 18, 1927, one specimen.

*Micromaseus patruelis* Dej.

At *Spartina* consocieties, 8 mi. southeast of Britt, May 19, 1928, one specimen.

*Amara impuncticollis* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 30, 1926, one specimen.

*Triaena pallipes* Kby.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 26, 1926, one specimen. On *Lespedeza capitata*, 2 mi. north of Ames, June 24, 1926, one specimen taken, several others seen.

*Triaena angustata* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, May 2, 1928; latest adult, July 6, 1928. Numerous at *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, 1928.

*Rembus expansa* Csy.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 21, June 5, 1927, two specimens.

*Rembus laticollis* Lec.

At *Spartina* consocieties, 2.5 mi. north of Ames, May 2, 1928, one specimen, under debris.

*Platynus decorus* Say

At *Polygonum amphibium* sociies, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Platynus atratus* Lec.

At *Spartina* consocieties, under debris, 2.5 mi. north of Ames, May 2, 1928, one specimen.

*Platynus placidus* Say

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 19, 1927, one specimen.

*Platynus aeruginosus* Dej.

At *Spartina* consocieties, 8 mi. southeast of Britt, Aug. 9, 1928, one specimen.

*Platynus lutulentus* Lec.

At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, July 6, 1928, one specimen.

*Leptotrachelus dorsalis* Fab.

At *Spartina* consocieties, Lake Amana, Aug. 12, 31, 1927, two specimens.

*Galerita janus* Fab.

At *Spartina* consocieties, under debris, 2.5 mi. north of Ames, May 7, 1928, one specimen.

*Lebia atriventris* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associes. Earliest adult, Aug. 5, 1927; latest adult, Aug. 25, 1928. Not numerous.

*Lebia viridis* Say

At all communities. Earliest adult, May 12, 1927; latest adult, Aug. 13, 1927. Most of the species were taken at *Spartina* consocieties, where it is a common ground beetle.

*Lebia pumila* Dej.

At all communities lower than *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, May 9, 1928; latest adult, Aug. 12, 1927. More specimens taken at *Andropogon furcatus*—*Spartina Michauxiana* associes than at any other community, and common at that community.

*Lebia pleuritica* Lec.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 26, 1926, one specimen. At *Spartina* consocieties, 1 mi. south of Amana, Aug. 12, 1927, and Gitchie-Manito State Park, July 24, 1928, two specimens.

*Lebia viridipennis* Dej.

At *Spartina* consocieties, 1 mi. south of Amana, Aug. 25, 1928, one specimen.

*Lebia scapularis* Dej.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 21, 1926, and 6 mi. northwest of Cedar Falls, July 17, 1926, two specimens. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 30, 1926, and 7.75 mi. northwest of Thompson, June 30, 1928, two specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Calleida punctata* Lec.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 20, 1925, one specimen. At *Spartina* consocieties, earliest adult, Aug. 2, 1926, and latest adult, Aug. 31, 1927. Common at *Spartina* consocieties.

*Cymindis pilosa* Say

At *Bouteloua hirsuta*—*B. curtipendula* association, closely grazed, Ocheyedan Mound, July 23, 1928, one specimen.

*Brachinus perplexus* Dej.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, and 2.5 mi. north of Ames, July 16, 1928, two specimens.

*Chlaenius diffinis* Chaud.

At *Spartina* consocieties, 2.5 mi. north of Ames, May 7, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Apr. 25, 1928, one specimen under a stone.

*Chlaenius laticollis* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 25, 1927, two specimens, under stones.

*Chlaenius sericeus* Forst.

At *Spartina* consocieties, under debris, 2.5 mi. north of Ames, May 7, 1928, one specimen.

*Harpalus erraticus* Say.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, one specimen, on the ground.

*Harpalus compar* Lee.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 9, 16, 1926. Both specimens were taken at night.

*Harpalus pennsylvanicus* De G.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, May 9, 1928, one specimen, under a stone.

*Harpalus pleuriticus* Kby.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 7, 1928, and 5 mi. south of Stanhope, May 9, 1928, two specimens, under stones. At *Spartina* consocieties, subterranean, 1 mi. south of Gruver, six specimens, 2.5 mi. south of Ames, Apr. 22, 1928, one specimen, and 2.5 mi. north of Ames, under debris, May 2, 1928, one specimen. Common at *Spartina* consocieties.

*Harpalus herbivagus* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 31, June 22, 1926, two specimens, 5 mi. south of Stanhope, under stones, Apr. 25, Oct. 20, 1928, two specimens, and June 15, 1928, one specimen taken by sweeping. At *Spartina* consocieties under debris, 2.5 mi. north of Ames, May 2, 1928, one specimen.

*Triplectrus rusticus* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 25, 1928, one specimen, under stone. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 28, July 15, 1928, two specimens.

*Pseudamphasia sericea* Harr.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, June 23, 1926; latest adult, July 15, 1928. Probably more numerous at *Spartina* consocieties than at any other community, and there the species is common.

*Stenolophus conjunctus* Say

Nearly all of the specimens were taken at *Andropogon furcatus*—*Spartina Michauxiana* associates. The species was common at *Andropogon furcatus* consocieties in early spring, but when standing water has disappeared from *Spartina* consocieties the species was prevalent there. Earliest adult, Apr. 12, 1928; latest adult, Sept. 15, 1928.

*Tachistodes partiaris* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 26, 1928, one specimen.

*Agonoderus pallipes* Fab.

At nearly all communities. Earliest adult, April 22, 1928; latest adult, June 30, 1928. Not numerous.

*Silpha inaequalis* Fab.

Under dead cottontail rabbit, 5 mi. south of Stanhope, May 5, 1928, one specimen.

*Choleva basillaris* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 26, 1926, one specimen.

*Stenus flavicornis* Er.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen.



*Xantholinus emmesus* Grav.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Oct. 20, 1928, one specimen, under stone.

*Tachyporus scitulus* Er.

At *Carex* socias, 1 mi. south of Amana, June 23, 1928, two specimens.

*Hister americanus* Payk.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, March 25, 1928, under stone; latest adult, May 20, 1927. Not numerous.

*Platysoma lecontei* Mars.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest Buffalo Center, May 18, 1929, several specimens.

*Lucidota nigricans* Say

Nearly all specimens taken at *Spartina* consocias, and *Andropogon furcatus* consocias. Earliest adult, May 19, 1928; latest adult, July 30, 1928. Common at *Andropogon furcatus* consocias, and *Spartina* consocias.

*Lucidota indicta* Lec.

At *Spartina* consocias and *Carex* socias. Earliest adult, June 23, 1928; latest adult, July 17, 1928. Not numerous, one to two at each of several stations.

*Pyraetomena angulata* Say

At *Andropogon furcatus* consocias, 2.5 mi. south of Ames, June 5, 1926, one specimen, and at *Carex* socias, 1 mi. south of Amana, June 23, 1928, one specimen.

*Chauliognathus pennsylvanicus* De G.

At all communities. Earliest adult, July 24, 1928; latest adult, Sept. 19, 1928. Common at *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon furcatus* consocias where herbaceous plants are more numerous. The species is most numerous at flowers of *Compositae* such as *Solidago* spp. and *Helianthus* spp.

*Podabrus tomentosus* Say

At *Andropogon furcatus* consocias. Earliest adult, May 31, 1926; latest adult, July 6, 1928. Not common.

*Cantharis tantillus* Lec.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 21, 1927; latest adult, June 30, 1928. Not numerous at any station, but probably more common at *Spartina* consocias than at any other community.

*Cantharis carolinus* Fab.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, May 25, 1926; latest adult, June 30, 1928. Not numerous at any community.

*Cantharis rectus* Melsh.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 30, 1927, one specimen.

*Cantharis flavipes* Lec.

At *Carex* societies, 1 mi. south of Amana, June 23, 1928, one specimen.

*Cantharis luteicollis* Germ.

At *Spartina* consocieties, Lake Amana, June 23, 1928, four specimens, and 2.5 mi. south of Ames, May 30, 1927, two specimens. At *Carex* societies, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Cantharis simpliunguis* Blatch.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 20, 1927, two specimens. At *Andropogon furcatus* consocieties, 5 mi. east of Renwick, May 19, 1928, three specimens.

*Ditemnus bidentatus* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 29, 31, 1925, and May 11, 21, 1926. Four specimens.

*Silis latilobus* Blatch.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, May 19, 1928; latest adult, Aug. 13, 1927. Most of the specimens are from *Spartina* consocieties, but not numerous at any station.

*Tryptherus latipennis* Germ.

Swept from flowers of *Rudbeckia hirta*, 1 mi. south of Amana, June 23, 1928, one specimen, and from flowers of *Anemone canadense*, same locality and date, one specimen.

*Collops tricolor* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 24-26, 1928. Common.

*Collops sublimbatus* Schaeff.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 26-30, 1928. Scarce.

*Collops quadrimaculatus* Fab.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, June 23, 1928; latest adult, Aug. 26, 1926. Most numerous at *Andropogon furcatus* consocieties, where it is common.

*Trichodes nutalli* Kby.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, one specimen.

*Hydnocera lecontei* Wole.

At all communities. Earliest adult, June 23, 1928; latest adult, Aug. 26, 1926. Not common at any community.

*Hydnocera tricondylae* Lec.

At *Andropogon furcatus* consociés, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, July 6, 1928; latest adult, Aug. 9, 1928. Not common at either community.

*Rhipiphorus dimidiatus* Fab.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, July 16, 1925, one specimen.

*Epicauta trichrus* Pall.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, July 7, 1928, and 7.75 mi. northwest of Thompson, June 30, 1928. Six specimens. At *Andropogon furcatus* consociés, 5 mi. east of Renwick, Aug. 9, 1928, one specimen. Common at *Stipa spartea*—*Andropogon scoparius* association.

*Epicauta sericans* Lec.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 mi. south of Westfield, July 26, 1928, one specimen, and Sergeant Bluff, July 26, 1928, two specimens.

*Epicauta lemniscata* Fab.

At *Spartina* consociés, Lake Amana, Aug. 12, 1927, two specimens.

*Epicauta cinerea* Forst.

At *Bouteloua hirsuta*—*B. curtipendula* association, Ochevedan Mound, July 23, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, three specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, and Gitchie-Manito State Park, July 24, 1928, two specimens.

*Epicauta marginata* Fab.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, Aug. 17, 1926, one specimen.

*Epicauta pennsylvanica* De G.

At all communities higher than *Spartina* consociés, and lower than *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, July 24, 1928; latest adult, Sept. 5, 1928. Numerous at *Stipa spartea*—*Bouteloua curtipendula* association, and *Andropogon furcatus* consociés where *Compositae*, in blossom, were common. *Solidago* spp. appeared to be most attractive to this most common blister beetle.

*Macrobasis unicolor* Kby.

At *Amorpha canescens*, 1.5 mi. northeast of Ochevedan, July 23, 1928, one specimen.

*Nemognatha sparsa* Lec.

At flower of *Dyssodia papposa*, Oak Grove State Park, July 25, 1928, two specimens.

*Notoxus anchora* Hentz

At *Andropogon furcatus*—*Spartina Michauxiana* associes. Earliest adult, June 9, 1926; latest adult, July 7, 1928. Scarce.

*Notoxus monodon* Fab.

At *Bouteloua hirsuta*—*B. curtipendula* association, 5 mi. south of Stanhope, Oct. 20, 1928, one specimen.

*Anthicus formicarius* Laf.

At *Andropogon furcatus* consocies. Earliest adult, Apr. 12, 1926; latest adult, Aug. 17, 1926. Scarce.

*Anthicus cervinus* Laf.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, Apr. 12, 1926, one specimen.

*Anthicus lutulentus* Csy.

At *Spartina* consocies, 2.5 mi. south of Ames, May 5, 1926, one specimen.

*Lacon rectangularis* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, one specimen, and under stone at *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, May 9, 1928, one specimen.

*Monocrepidius vespertinus* Fab.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, Aug. 16, 1926, and at *Spartina* consocies, 10 mi. southwest of Kelso, July 30, 1928.

*Monocrepidius auritus* Hbst.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, June 19, 1926, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 5, 1927, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, two specimens.

*Drasterius elegans* Fab.

At *Spartina* consocies, 2.5 mi. south of Ames, May 12, 1926. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 25, 1927, one specimen, and 5 mi. south of Stanhope, May 9, 1928, one specimen, under stone.

*Limonijs propexus* Cand.

At *Andropogon furcatus*—*Spartina Michauxiana* associes, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 9, 1928; latest adult, July 6, 1928. A common click beetle of which most specimens have come from *Andropogon furcatus* consocies.

*Ludius inflatus* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associes, 2.5 mi. south of Ames, May 28, 29, 1926, and June 5, 1927. Three specimens.



*Hemicrepidius memnonius* Hbst.

At *Spartina* consocieties, subterranean, 1 mi. south of Gruver, July 8, 1928, one specimen.

*Hemicrepidius bilobatus* Say

At *Spartina* consocieties, subterranean, 1 mi. south of Gruver, July 8, 1928, one specimen.

*Cryptohypnus abbreviatus* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 30, 1926, one specimen.

*Oedostethus femoralis* Lec.

At *Carex* sociies, 1 mi. south of Amana, June 23, 1928, three specimens.  
At *Stipa spartea*—*Bouteloua curtipendula* association, 5 mi. south of Stanhope, June 15, 1928, one specimen.

*Agriotes oblongicollis* Melsh.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Melanotus cribulosus* Lec.

At *Andropogon furcatus* consocieties, and *Spartina* consocieties. Earliest adult, May 26, 1926; latest adult, Aug. 5, 1927. A common click beetle at *Andropogon furcatus* consocieties.

*Acmaeodera pulchella* Hbst.

At flowers of *Rudbeckia hirta*, 5 mi. east of Renwick, July 8, 1928, and at several species of *Compositae* flowers, 2.5 mi. south of Ames, July 10, 1928.

*Agrilus lacustris* Lec.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Pachyschelus purpureus* Say

On *Desmodium illinoense* leaves, 2.5 mi. south of Ames, July 20, 1925, one specimen, and 6 mi. northwest of Ledyard, July 7, 1928, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10, 1925, one specimen.

*Taphrocerus gracilis* Say

At *Spartina* consocieties, 2.5 mi. south of Ames, May 21, 1927, and Lake Amana, Aug. 12, 1927. Two specimens.

*Ptilodactyla serricollis* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 23, 1926, two specimens.

*Dermestes caninus* Germ.

Under dead jackrabbit, Ocheyedan Mound, July 23, 1928, two specimens.

*Brachypterus urticae* Fab.

At *Stipa spartea*—*Andropogon scoparius* association, northeast of Iowa State College grounds, July 11, 1928, one specimen.

*Conotelus obscurus* Er.

In flowers of *Convolvulus sepium*, 1 mi. south of Amana, July 20, 1928, three specimens.

*Carpophilus dimidiatus* Fab.

Swept from *Amorpha canescens*, 1.5 mi. northeast of Ocheyedon, July 23, 1928, one specimen.

*Carpophilus brachypterus* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 9, 1928, in *Anemone patens* var. *Wolfgangiana* flower, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, earliest adult, Apr. 26, 1926, and latest adult, July 10, 1926. Common at the last community.

*Carpophilus antiquus* Melsh.

At *Spartina* consocieties, Lake Amana, June 23, 1928, two specimens.

*Epuraea rufa* Say

At *Spartina* consocieties, 2.5 mi. south of Ames, May 12, 1927, one specimen.

*Glischrochilus fasciatus* Oliv.

At *Pedicularis canadensis*, blooming, 2.5 mi. south of Ames, May 17, 1926, one specimen. At *Andropogon furcatus* consocieties, same locality, May 20, 1927, one specimen.

*Telephanus velox* Hald.

At *Spartina* consocieties, Lake Amana, Aug. 12, 1927, one specimen.

*Languria bicolor* Fab.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 23, 26, 1926, two specimens.

*Languria mozardi* Latr.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Apr. 16, 1927; latest adult, Aug. 17, 1926. Common at *Andropogon furcatus* consocieties.

*Atomaria ephippiata* Zimm.

At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, May 19, 1928, one specimen.

*Typhaea fumata* Linn.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Phalacrus simplex* Lee.

At *Andropogon furcatus*—*Spartina Michauxiana* associes, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 18, 1928; latest adult, July 23, 1928. Scarce.

*Phalacrus politus* Melsh.

At *Andropogon furcatus*—*Spartina Michauxiana* associes, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 19, 1928; latest adult, Sept. 19, 1928. At flowers of *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927. Common.

*Olibrus semistriatus* Lec.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, May 12, 1927, one specimen.

*Olibrus pallipes* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association. Gitchie-Manito State Park, July 24, 1928, one specimen.

*Stilbus apicalis* Melsh.

At *Spartina* consocies, .5 mi. south of Missouri Valley, Aug. 1, 1928, one specimen.

*Scymnus americanus* Muls.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, Aug. 17, 1926, one specimen.

*Hyperaspis undulata* Say

At nearly all communities lower than *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, May 7, 1928; latest adult, Aug. 9, 1928. Common at *Andropogon furcatus* consocies.

*Coccidula lepida* Lec.

At *Spartina* consocies. Earliest adult, May 18, 1928; latest adult, June 23, 1928. Common.

*Anisosticta strigata* Thunb.

At *Spartina* consocies, 4 mi. northwest of Thompson, May 18, 1928, one specimen.

*Macronaemia episcopalis* Kby.

At *Spartina* consocies, 4 mi. northwest of Thompson, May 18, 1928, one specimen.

*Megilla maculata* De G.

At *Spartina* consocies, chiefly. Earliest adult, May 5, 1926. Latest adult, Oct. 20, 1928, at *Stipa spartea*—*Bouteloua curtipendula* association. 5 mi. south of Stanhope. Common at *Spartina* consocies.

*Hippodamia tredecim-punctata* Linn.

At all communities. Earliest adult, May 7, 1928; latest adult, Oct. 20, 1928. Common.

*Hippodamia parenthesis* Say

At all communities. Earliest adult, May 2, 1928; latest adult, Oct. 20, 1928. Common.

*Hippodamia glacialis* Fabr.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, 1928, two specimens. At *Andropogon furcatus* consocieties, 6 miles northwest of Cedar Falls, July 17, 1926, one specimen, and 3.5 mi. north of Ledyard, July 7, Aug. 7, 1928, two specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Giethie-Manito State Park, July 24, 1928.

*Hippodamia convergens* Guer.

At all communities lower than *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, June 30, 1928; latest adult, Sept. 7, 1928. Common.

*Coccinella trifasciata* Linn.

At *Elymus virginicus*, 1 mi. south of Amana, Aug. 12, 1927, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedan Mound, July 23, 1928, two specimens.

*Coccinella novemnotata* Hbst.

At all communities lower than *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, May 20, 1927; latest adult, Aug. 25, 1928. Common.

*Coccinella sanguinea* Linn.

At all communities lower than *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, July 11, 1928; latest adult, Sept. 7, 1928. Common at *Andropogon furcatus* consocieties, and *Andropogon furcatus*—*Sorghastrum nutans* associates.

*Eleodes tricolorata* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. north of Reels City, Aug. 1, 1928, one specimen.

*Ptinus brunneus* Duft.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Mar. 25, 1928, one specimen.

*Canthon laevis* Dru.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, May 9, 1928, one specimen under a stone.

*Onthophagus hecate* Panz.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 9, 1928; latest adult, Aug. 7, 1925. Under dead cottontail rabbit, 5 mi. south of Stanhope, May 9, 1928, four specimens. Not numerous.

*Aphodius fimetarius* Linn.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 16, 1927, one specimen.



*Aphodius distinctus* Mull.

At all communities except *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, Mar. 25, 1928; latest adult, Oct. 20, 1928. Common.

*Aphodius alternatus* Horn

At *Andropogon furcatus* consocieties, 2 mi. south of Ledyard, May 9, 1926, four specimens. At *Spartina* consocieties, 4 mi. northwest of Thompson, May 18, 1928, and 8 mi. southeast of Britt, May 19, 1928, two specimens.

*Aphodius socialis* Brown

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Oct. 20, 1928, one specimen.

*Ataenius cognatus* Lec.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 12, 1927, one specimen.

*Bolbocerosoma farctum* Fab.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, June 25, 1925, and June 23, 26, 1926. Three specimens.

*Serica* sp.

At *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 30, 1928; latest adult, July 7, 1928. Common at *Amorpha canescens*, 7.75 mi. northwest of Thompson, June 30, 1928.

*Phyllophaga* sp.

At *Spartina* consocieties, subterranean, 2 mi. west of Kelso, July 30, 1928, one specimen.

*Anomala innuba* Fab.

Swept from *Apocynum androsacmifolium*, 1 mi. south of Amana, June 23, 1928, two specimens.

*Ligyrodes relictus* Say

At *Spartina* consocieties, subterranean, 1 mi. south of Gruver, July 8, 1928, four specimens, 10 mi. southwest of Kelso, July 30, 1928, one specimen, and 2 mi. west of Kelso, July 30, 1928, one specimen.

*Ligyrys gibbosus* De G.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, March 25, 1928, one specimen, under stone.

*Euphoria inda* Linn.

At *Spartina* consocieties, 2.5 mi. south of Ames, Mar. 17, 1928, one specimen.

*Typocerus confluens* Csy.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 20, 1928, two specimens.

*Typocerus sinuatus* Newm.

At *Andropogon furcatus* consocieties, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, July 7, 1928; latest adult, Aug. 7, 1928. Common at flowers of *Compositae* of *Andropogon furcatus* consocieties.

*Mecas saturnina* Lec.

Swept from *Lepachys pinnata*, 2.5 mi. south of Ames, July 15, 1928, one specimen.

*Oberea tripunctata* Swed.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, July 20, 1928, one specimen.

*Tetraopes tetrophthalmus iowensis* Csy.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, July 6, Aug. 26, 1926, three specimens on *Asclepias* sp. At *Carex* societies, Lake Amana, July 23, 1928, one specimen.

*Tetraopes femoratus amnicola* Csy.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, and 4 mi. northeast of Beloit, July 25, 1928, four specimens. At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedan Mound, July 23, 1928, one specimen.

*Lema brunnicollis* Lac.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Lema collaris* Say

Cut out of stems of *Tradescantia reflexa*, 1 mi. south of Amana, July 20, 1928, two specimens which had emerged from puparia within stems. Several other pupae were found in the stems.

*Lema trilineata* Oliv.

At *Stipa spartea*—*Andropogon scoparius* association, northeast of Iowa State College grounds, July 11, 1928, one specimen, and at *Andropogon furcatus* consocieties, 2 mi. south of Ledyard, May 9, 1926, one specimen.

*Antipus laticlavus* Forst.

At *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 15, 1928; latest adult, June 30, 1928. Common on *Amorpha canescens*, 7.75 mi. northwest of Thompson, June 30, 1928.

*Coscinoptera dominicana* Fab.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Coscinoptera* near *axillaris* Lec.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, one specimen.

*Babia quadriguttata* Oliv.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 1, 1926, one specimen.

*Pachybrachys spumarius* Suffr.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 16, 1925, one specimen.

*Pachybrachys othonus* Say

At all communities except *Bouteloua hirsuta*—*B. curtispindula* association. Earliest adult, June 5, 1926; latest adult, July 30, 1928. Not numerous at any station.

*Pachybrachys m-nigrum* Melsh.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, 1928, one specimen.

*Pachybrachys luridus* Fab.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, June 15, 1928, five specimens, of which two were taken on *Amorpha canescens*.

*Monachulus ater* Hald.

At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, July 6, 1928, one specimen.

*Monachus saponatus* Fab.

At *Spartina* consocieties, and *Carex* societies, chiefly. Earliest adult, June 30, 1927; latest adult, Aug. 5, 1927. Not common.

*Cryptocephalus leucomelas* Suffr.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, July 20, 1928, one specimen.

*Cryptocephalus venustus* Fab.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10, 1925, and July 2, 1926, two specimens. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, 1928, two specimens.

*Cryptocephalus venustus cinctipennis* Rand.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. *Andropogon furcatus*—*Sorghastrum nutans* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 5, 1928; latest adult, Aug. 14, 1925. Not numerous.

*Cryptocephalus venustus hamatus* Melsh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, *Andropogon furcatus*—*Sorghastrum nutans* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 23, 1928; latest adult, July 29, 1925. Not numerous.

*Cryptocephalus incertus* Hald.

Swept from *Senecio aureus*, 1 mi. south of Amana, June 23, 1928, one specimen. At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, July 6, 1928, one specimen. Swept from *Psoralea argophylla*, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, and Oak Grove State Park, July 25, 1928, two specimens.

*Cryptocephalus calidus* Suffr.

Feeding on leaflet of *Amorpha canescens*, 1 mi. south of Amana, July 20, 1928, one specimen. On *Helianthus grosseserratus*, same locality, Aug. 12, 1927, several specimens. At *Andropogon furcatus* consocieties, earliest adult, July 7, 1928, and latest adult, Aug. 9, 1928. At *Stipa spartea*—*Andropogon scoparius* association, 1.5 mi. northeast of Ochevedan, July 23, 1928, and 7.75 mi. northwest of Thompson, two specimens. Not numerous.

*Bassareus lituratus* var. *recurvus* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associes, 2.5 mi. south of Ames, May 21, 1926, June 5, 1927, two specimens.

*Nodonota tristis* Oliv.

At *Andropogon furcatus*—*Sorghastrum nutans* associes, 1 mi. south of Amana, June 23, 1928. At *Stipa spartea*—*Andropogon scoparius* association, earliest adult, June 30, 1928, and latest adult, July 23, 1928. Common at *Andropogon scoparius*—*Bouteloua curtipendula* association, July 24-26, 1928.

*Nodonota clypealis* Horn

At *Polygonum amphibium* socies, 10 mi. southwest of Kelso, July 30, 1928, one specimen.

*Nodonota convexa* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associes, *Andropogon furcatus*—*Sorghastrum nutans* associes, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 26, 1926; latest adult Aug. 12, 1928. Most numerous at flowers of *Zizia aurea* and *Erigeron ramosus*, 1 mi. south of Amana, June 23, 1928.

*Nodonota puncticollis* Say

At *Andropogon furcatus* consocieties, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 1, 1926; latest adult, July 23, 1928. Most numerous on *Amorpha canescens*, 7.75 mi. northwest of Thompson, June 30, 1928.

*Colaspis brunnea* Fab.

At all communities higher than *Spartina* consocieties. Earliest adult, July 7, 1928; latest adult, Aug. 26, 1928. One adult fed on leaf of *Helianthus grosseserratus*, 1 mi. south of Amana, Aug. 13, 1927. Numerous on *Aster multiflorus*, 1.5 mi. northeast of Ochevedan, July 23, 1928.

*Colaspis favosa* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associes. Earliest adult, June 23, 1928; latest adult, Aug. 26, 1926. Most numerous at *Spar-*



*tina* consociés, and *Polygonum amphibium* sociés. An adult was observed feeding on a leaf of *Polygonum amphibium*, 2.5 mi. south of Amana, July 13, 1926.

*Rhabdopterus picipes* Oliv.

At *Andropogon furcatus*—*Spartina Michauxiana* associés. Earliest adult, June 23, 1926; latest adult, Aug. 12, 1927. Not common.

*Graphops varians* Lec.

At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Common at the latter association. Earliest adult, July 7, 1928; latest adult, Aug. 6, 1928.

*Graphops pubescens* Melsh.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, May 21, 1926, one specimen. Four specimens that were feeding on leaves of *Oenothera biennis* were taken 2.5 mi. south of Ames, June 5, 1926. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 12, 1927, one specimen.

*Graphops curtipennis* Melsh.

At *Andropogon furcatus* consociés, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 9, 1928; latest adult, Oct. 20, 1928. Common.

*Graphops marcassitus* Cr.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, Aug. 9, 1926, one specimen, and 3.5 mi. north of Ledyard, Aug. 7, 1928, one specimen.

*Metachroma interruptum* Say

On *Helianthus grosseserratus*, Lake Amana, June 23, 1928, one specimen.

*Myochrous squamosus* Lec.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Paria canella* Fab.

At *Spartina* consociés, 2.5 mi. south of Ames, May 5, 1926, two specimens.

*Paria canella aterrima* Oliv.

At *Andropogon furcatus*—*Spartina Michauxiana* associés, *Andropogon furcatus*—*Sorghastrum nutans* associés, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 23, 1928; latest adult, Aug. 13, 1927. Common at *Andropogon furcatus*—*Sorghastrum nutans* associés, and *Spartina* consociés, 1 mi. south of Amana.

*Paria canella* var. near *pumila* Lec.

At *Spartina* consociés, 8 mi. southeast of Britt, May 19, 1928, one specimen.

*Paria canella quadriguttata* Lec.

Swept from *Anemone canadensis*, 1 mi. south of Amana, June 23, 1928, one specimen.

*Paria canella quadrinotata* Say

At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon furcatus*—*Spartina Michauxiana* associes. Earliest adult, May 9, 1926; latest adult, July 10, 1928. Not numerous at any community but probably more frequent at *Spartina* consocies than at any other community.

*Paria canella sellata* Horn

At *Andropogon furcatus*—*Sorghastrum nutans* associes, 1 mi. south of Amana, June 23, 1928, eight specimens.

*Paria canella sexnotata* Say

At *Spartina* consocies, 2.5 mi. south of Ames, May 30, 1927, one specimen.

*Chrysochus auratus* Fab.

On *Apocynum androsaemifolium*, 2.5 mi. south of Ames, numerous. Earliest adult, July 22, 1925; latest adult, Aug. 11, 1925. At *Andropogon furcatus*—*Sorghastrum nutans* associes, 1 mi. south of Amana, June 23, 1928, one specimen.

*Prasocuris vittata* Oliv.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, May 12, 1927, one specimen.

*Labidomera clivicollis* var. *rogersi* Lec.

Swept from *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927, two specimens.

*Zygogramma suturalis* Fab.

At *Spartina* consocies, 1 mi. south of Amana, Aug. 13, 1927, one specimen.

*Zygogramma suturalis* var. *casta* Rog.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, three specimens. On flower of *Lepachys pinnata*, 5 mi. south of Stanhope, Aug. 5, 1927, one specimen.

*Calligrapha similis* Rog.

At *Spartina* consocies, 1 mi. south of Amana, and Lake Amana, Aug. 12, 13, 1927, three specimens.

*Calligrapha praeclis* Rog.

At *Spartina* consocies, Lake Amana, June 23, 1928, one specimen.

*Phaedon viridis* Melsh.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, May 12, 1927, one specimen.

*Gastroidea dissimilis* Say

At *Polygonum amphibium* societies, .5 mi. south of Missouri Valley, Aug. 1, 1928, three specimens.

*Lina scripta* Fab.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Trirhabda virgata* Lec.

At all communities. Earliest adult, July 7, 1928; latest adult, Sept. 19, 1928. Numerous on *Helianthus* spp. and *Solidago* spp. feeding on leaves.

*Galerucella americana* Fab.

At *Andropogon furcatus* consocieties, *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, June 30, 1928; latest adult, Aug. 9, 1928. Not common at any station.

*Galerucella conferta* Lec.

At *Spartina* consocieties, Lake Amana, Aug. 12, 1927, June 23, 1928, five specimens. At *Carex* societies, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Galerucella cribrata* Lec.

At *Stipa spartea*—*Andropogon scoparius* association, chiefly. Earliest adult, June 30, 1928; latest adult, Aug. 20, 1928. Not numerous.

*Galerucella decora* Say

At *Andropogon scoparius*, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen, and *Carex* societies, same locality and date, one specimen.

*Diabrotica duodecimpunctata* Fabr.

At all communities except *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, May 21, 1927; latest adult, Sept. 19, 1928. Common at flowers of *Compositae*, especially in August and September.

*Diabrotica longicornis* Say

At all communities except *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, Aug. 14, 1926; latest adult, Oct. 20, 1928. Numerous at flowers of *Compositae*, August and September.

*Diabrotica atripennis fossata* Lec.

At *Polygonum amphibium* societies, 2.5 mi. south of Ames, July 22-Aug. 9, 1926, common, feeding on leaves of *P. amphibium*. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, two specimens.

*Diabrotica vittata* Fab.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 21, 1926, one specimen.

*Phyllobrotica decorata* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 30, 1926, and 6 mi. northwest of Cedar Falls, July 17, 1926. At *Spartina* consocieties, Lake Amana, June 23, 1928. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928. One specimen on each date.

*Phyllobrotica limbata* Fab.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 19, 26, 1926, two specimens.

*Cerotoma trifurcata* Forst.

At *Andropogon furcatus* consocieties, and *Spartina* consocieties. Earliest adult, June 23, 1926; latest adult, Aug. 12, 1927. Scarce.

*Hypolampsis pilosa* Ill.

At *Stipa spartea*—*Andropogon scoparius* association, 6 mi. northwest of Ledyard, Aug. 7, 1928, one specimen.

*Oedionychis gibbitarsa* Say.

At *Spartina* consocieties, under debris, 2.5 mi. north of Ames, May 2, 1928.

*Oedionychis miniata* Fab.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, three specimens.

*Oedionychis thyamoides* Cr.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, *Andropogon furcatus*—*Sorghastrum nutans* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 8, 1926; latest adult, Sept. 7, 1927. One adult was seen feeding on leaf of *Silphium laciniatum*, 2.5 mi. south of Ames, May 19, 1926. Not numerous.

*Oedionychis limbalis* Melsh.

At *Spartina* consocieties, 4 mi. northwest of Thompson, May 18, 1928, and at *Andropogon furcatus* consocieties, 5 mi. east of Renwick, May 19, 1928, one specimen.

*Disonycha triangularis* Say

At *Polygonum amphibium* societies, 2.5 mi. south of Ames, Aug. 2, 1926, and 10 mi. southwest of Kelso, two specimens. At *Stipa spartea*—*Andropogon scoparius* association, 1.5 mi. northwest of Ocheyedan, July 23, 1928, one specimen.

*Disonycha xanthomelaena* Dalm.

At *Andropogon furcatus* consocieties, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, Apr. 24, 1927; latest adult, July 31, 1928. Scarce.

*Haltica litigata* Fall

At *Spartina* consocieties, 2.5 mi. south of Ames, May 30-June 5, 1927. Not numerous.



*Haltica foliacea* Lec.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Chalcoides fulvicornis nana* Say

At *Spartina* consocieties, and *Carex* societies. Earliest adult, June 30, 1928; latest adult, Sept. 15, 1928. Common at *Spartina* consocieties, 7.75 mi. northwest of Thompson, Sept. 15, 1928.

*Epitrix fuscula* Cr.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 30, 1927, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Epitrix parvula* Fab.

Swept from blooming *Rosa pratincola* 1 mi. south of Amana, June 23, 1928, one specimen.

*Chaetocnema denticulata* Ill.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10, 29, 1925, two specimens.

*Chaetocnema opulenta* (?) Horn

At *Andropogon furcatus* consocieties, 6 mi. northwest of Le Mars, July 26, 1928, one specimen.

*Systema frontalis* Fab.

At *Polygonum amphibium* consocieties, earliest adult, July 26, 1926; latest adult, Sept. 16, 1928. Common. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Systema elongata* Fab.

At *Andropogon furcatus* consocieties, chiefly. Earliest adult, Aug. 6, 1928; latest adult, Sept. 15, 1928. Common.

*Longitarsus testaceus* Melsh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Apr. 26, 1926; latest adult, Aug. 11, 1925. Common. In flower of *Pedicularis canadensis*, 2.5 mi. south of Amana, May 12, 1926, one specimen.

*Glyptina spuria* Lec.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, Mar. 25, 1928; latest adult, Sept. 15, 1928. Not numerous.

*Phyllotreta zimmermanni* Cr.

At *Spartina* consocieties, Lake Amana, June 23, 1928, one specimen, and 8 mi. southeast of Britt, May 19, 1928, one specimen.

*Phyllotreta vittata* Fab.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10, 1925, one specimen, and June 22, 1926, one specimen.

*Phylloreta bipustulata* Fab.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Apr. 22, 1928; latest adult, Aug. 31, 1927. Not numerous.

*Psylliodes punctulata* Melsh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Bouteloua curtipendula* association. Earliest adult, Mar. 25, 1928; latest adult, Aug. 9, 1928. Common.

*Stenispa metallica* Fab.

At *Spartina* consociates and *Carex* societies, Lake Amana, June 23, Aug. 31, 1928, three specimens.

*Microrhopala vittata* Fabr.

At *Andropogon furcatus* consociates, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, May 5, 1926; latest adult, Aug. 26, 1925. Larvae, leaf miners of *Silphium laciniatum*, were frequently seen. Common where *S. laciniatum* occurs in families as at 2.5 mi. south of Ames.

*Chelymorpha cassidea* Fab.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, July 20, Aug. 25, 1928, two specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, two specimens.

*Jonthonota nigripes* Oliv.

At *Andropogon furcatus* consociates, 2.5 mi. south of Ames, Aug. 19, 1925, and June 1, 1926, two specimens.

*Chirida guttata* Oliv.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, *Andropogon furcatus*—*Sorghastrum nutans* associates, *Spartina* consociates, and *Carex* societies. Earliest adult, July 20, 1928; latest adult, Aug. 13, 1927. Not numerous.

*Metriona bivittata* Say

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, May 9, 1926; latest adult, Aug. 17, 1926. Scarce.

*Metriona bicolor* Fab.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, and *Andropogon furcatus* consociates. Earliest adult, June 18, 1925; latest adult, Sept. 16, 1928. Not numerous.

*Bruchus cruentatus* Horn

At *Polygonum amphibium* societies, 10 mi. southwest of Kelso, July 30, 1928, three specimens.

*Bruchus fraterculus* Horn

At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, June 30, 1928; latest adult, Aug. 6, 1928. Common at the first mentioned association.

*Bruchus musculus* Say

At *Andropogon furcatus* consociés. Earliest adult, June 30, 1928; latest adult, Aug. 9, 1928. Not numerous.

*Bruchus seminulum* Horn

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park to Sergeant Bluff, July 26, 30, 1928, three specimens.

*Brachytarsus sticticus* Boh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Apr. 30, 1926; latest adult, May 30, 1927. Common. Numerous at *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927.

*Brachytarsus tomentosus* Say

At *Stipa spartea*—*Andropogon scoparius* association, 3.5 mi. north of Ledyard, Aug. 7, 1928, one specimen.

*Rhynchites bicolor* Fab.

At *Rosa pratincola* flowers, 1 mi. south of Amana, June 23, 1928, 5 mi. south of Stanhope, June 15, 1928, and 7.75 mi. northwest of Thompson, June 30, 1928. Common at several stations.

*Rhynchites aeneus* Boh.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1926, one specimen.

*Apion pennsylvanicum* Boh.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1927, one adult.

*Apion tenuirostrum* Smith

At *Andropogon furcatus* consociés, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 19, 1928; latest adult, July 23, 1928. Not numerous.

*Apion griseum* Smith

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 9, 1926; latest adult, July 30, 1928. Not numerous.

*Apion varicorne* Smith

At *Andropogon furcatus* consociés, *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 19, 1928; latest adult, Sept. 15, 1928. Common at *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, Aug. 6, 1928.

*Apion* sp. 1

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Sept. 7, 1927, one specimen.

*Apion* sp. 2

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 16, 1927, four specimens.

*Apion* sp. 3

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 19, 1928; latest adult, Sept. 15, 1928. Six specimens.

*Apion* sp. 4

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Apr. 12, 1926; latest adult, July 22, 1925. Six specimens.

*Anametis granulata* Say

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, earliest date, Apr. 25, 1928, and latest date, Aug. 19, 1927. Swept from *Amorpha canescens*, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen, and from *Helianthus occidentale*, 5 mi. south of Stanhope, Aug. 19, 1927. Numerous 5 mi. south of Stanhope, May 9, 1928.

*Tanymecus confertus* Gyll.

At *Spartina* consocieties, Lake Amana, Aug. 12, 1927, one specimen.

*Mesagroicus minor* Buch. (MS)

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Amana, May 20, 1927, and June 26, 1926. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, June 15, 1928. Three specimens, in total. This is a new species named by L. L. Buchanan, and was in manuscript at the time of return of the specimens, December, 1928.

*Graphorhinus vadosus* Say

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, one specimen.

*Epicaerus imbricatus* Say

Swept from *Elymus virginicus*, 1 mi. south of Amana, Aug. 12, 1927, one specimen, and *Andropogon furcatus*, 1.5 mi. east of Verdi, Aug. 20, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua* association, earliest adult, May 9, 1928, and latest adult, Aug. 6, 1928. Common at *Amorpha canescens* families.

*Lepidocricus herricki* Pierce

Taken on flower of *Fragaria virginiana*, 2.5 mi. south of Ames, May 12, 1926, one specimen.

*Brachyrhinus ovatus* Linn.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Aphrastus taeniatus* Gyll.

At *Andropogon furcatus* consocieties, 6 mi. northwest of Cedar Falls, July 17, 1928, one specimen.



*Sitona flavescens* Marsh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, Aug. 7, 1925, and 6 mi. northwest of Ledyard, Aug. 7, 1928. Four specimens.

*Sitona hispidula* Germ.

At *Spartina* consociates. Earliest adult, Aug. 12, 1927; latest adult, Aug. 31, 1927. Not numerous.

*Sitona* (?) *lineellus* Gyll.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, Aug. 6, 1928, and 6 mi. northwest of Ledyard, Aug. 7, 1928. Two specimens.

*Sitona tibialis* Hbst.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 9, 1926; latest adult, Aug. 6, 1926. Common at *Stipa spartea*—*Andropogon scoparius* association.

*Hypera punctata* Fab.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, Aug. 7, 1928; latest adult, Sept. 1, 1928. Probably common at *Spartina* consociates.

*Phytonomus nigrirostris* Fab.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Apr. 16, 1927; latest adult, Aug. 17, 1925. Not numerous.

*Listronotus latiusculus* Boh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, June 5, 1927; latest adult, Aug. 11, 1925. Scarce.

*Hyperodes montanus* Dtz.

At *Spartina* consociates, earliest adult, May 19, 1928, and latest adult, July 11, 1928. Not numerous.

*Hyperodes delumbis* Gyll.

At *Andropogon furcatus* consociates, 5 mi. east of Renwick, May 19, 1928, two specimens.

*Hyperodes sparsus* Say

At *Andropogon furcatus* consociates, 1 mi. southwest of Ames, July 14, 1928, and 2 mi. south of Ledyard, May 9, 1926. Two specimens.

*Hyperodes obscurellus* Dtz.

At *Andropogon furcatus* consociates, 2 mi. south of Ledyard, May 9, 1926, one specimen.

*Notaris bimaculatus* Fab.

At *Phragmites communis*, 5 mi. northwest of Buffalo Center, Aug. 6, 1928, two specimens.

*Pachyphanes discoideus* Lec.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen. At *Spartina* consocieties, Lake Amana, Aug. 12, 1927, one specimen.

*Desmoris pervisus* Dtz.

Taken on flower bud of *Helianthus grosseserratus*, 2.5 mi. south of Ames, Aug. 26, 1925, one specimen.

*Desmoris constrictus* Say

On *Helianthus grosseserratus*, 1 mi. south of Amana, Aug. 13, 1927, two specimens, and 6 mi. northwest of Ledyard, Aug. 7, 1928, three specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park to Sergeant Bluff, July 25-26, 1928.

*Smicronyx corniculatus* Fahr.

At *Stipa spartea*—*Andropogon scoparius* association, northeast of Iowa State College grounds, July 11, 1928, one specimen.

*Endalus ovalis* Lec.

At *Andropogon furcatus* consocieties, 5 mi. east of Renwick, May 19, 1928, one specimen, and at *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Tanysphyrus lemnae* Fab.

At *Spartina* consocieties, 8 mi. southeast of Britt, May 19, 1928, one specimen.

*Bagous* sp.

At *Andropogon furcatus*—*Spartina Michauxiana* associations, 2.5 mi. south of Ames, June 26, 1926, one specimen, and 5 mi. east of Renwick, May 19, 1928, one specimen.

*Bagous restrictus* Lec.

At *Spartina* consocieties, 8 mi. southeast of Britt, one specimen.

*Thysanocnemis helvola* Lec.

At *Andropogon furcatus* consocieties, 6 mi. northwest of Le Mars, July 26, 1928, one specimen.

*Tychius tectus* Lec.

At *Andropogon furcatus* consocieties, 2 mi. south of Ledyard, May 9, 1926, one specimen, and 6 mi. northwest of Le Mars, July 26, 1928, four specimens.

*Anthonomus* (?) *virgo* Dtz.

At flowers of *Cassia Chamaecrista*, 1 mi. south of Amana, Aug. 25, 1928, two specimens.

*Anthonomus squamosus* Lec.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito and Oak Grove State Parks, July 24, 25, 1928. Two of the seven specimens were taken at flowers of *Dyssodia papposa*.

*Anthonomus rufipes* Lec.

At *Andropogon furcatus* consociés, 5 mi. east of Renwick, May 19, 1928, two specimens.

*Lixus mucidus* Lec.

All specimens taken from *Polygonum amphibium* sociés, a *Polygonum* sp. family, or a community containing a *Polygonum* sp. Earliest adult, June 26, 1926; latest adult, Sept. 16, 1926. Probably common at *Polygonum amphibium* sociés, and *Polygonum muhlenbergii* family.

*Lixus fimbriolatus* Boh.

At *Carex* sociés, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Lixus nitidulus* Csy.

At *Carex* sociés, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Lixus terminalis* Lec.

Cut out of stems of *Polygonum pennsylvanicum* in which the larvae had grown and pupated, 2 mi. west of Kelso, July 30, 1928. Numerous. At *Polygonum amphibium* sociés, earliest adult, June 26, 1926, and latest adult, Aug. 1, 1928.

*Lixus* sp. 1

At *Spartina* consociés, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Lixus* sp. 2

At *Andropogon scoparius*, 7.75 mi. northwest of Thompson, Aug. 6, 1928, one specimen. A second specimen came from a pupa found in the lower part of a stem of *Cicuta maculata*, 7.75 mi. northwest of Thompson. The pupa stage was secured Aug. 6, and the adult appeared Aug. 7, 1928.

*Baris striata* Say

Swept from *Astragalus caryocarpis* family, 5 mi. south of Stanhope, June 15, 1928, two specimens, and from *Amorpha canescens*, same locality and date, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedon Mound, July 23, 1928, and 7.75 mi. northwest of Thompson, June 30, 1928, two specimens.

*Baris transversa* Say

At *Andropogon furcatus*—*Sorghastrum nutans* associés, 1 mi. south of Amana, July 20, 1928, two specimens.

*Baris deformis* Csy.

At *Andropogon furcatus* consociés. Earliest adult, May 12, 1927; latest adult, May 19, 1928. Common.

*Aulobaris naso* Lec.

At *Andropogon furcatus*—*Sorghastrum nutans* association, 1.5 mi. east of Museatine, Sept. 1, 1928, and 1.5 mi. east of Verdi, Aug. 20, 1928. One specimen on each date.

*Pseudobaris nigrina* Say

At *Andropogon furcatus* consociés, 2 mi. south of Ledyard, May 9, 1928, one specimen.

*Centrinus penicellus* Hbst.

At *Polygonum amphibium* sociés, 10 mi. southwest of Kelso, July 30, 1928, one specimen, and at *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Odontocorynus salebrosus* Csy.

On flowers of *Erigeron ramosus*, and *Rudbeckia hirta*, chiefly, at *Andropogon furcatus* consociés, and *Andropogon furcatus*—*Sorghastrum nutans* associés. Earliest adult, June 23, 1928; latest adult, July 8, 1928. Common.

*Anacentrus deplanatus* Csy.

At *Spartina* consociés, 8 mi. southeast of Britt, May 19, 1928, one specimen.

*Cylindrocopturus operculatus* Say

Swept from *Helianthus Maximiliani*, 10 mi. southwest of Kelso, July 30, 1928, one specimen.

*Cylindrocopturus sparsus* Csy.

At *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927, one specimen.

*Ceutorhynchus rapae* Gyll.

Swept from flowers of *Cicuta maculata*, 2.5 mi. south of Ames, July 10, 1928, one specimen.

*Ceutorhynchus sulcipennis* Lec.

At *Polygonum amphibium* sociés, chiefly. Earliest adult, May 12, 1926; latest adult, Aug. 14, 1926. Numerous.

*Ceutorhynchus cyanipennis* Germ.

At *Andropogon furcatus* consociés, 5 mi. east of Renwick, May 19, 1928, one specimen.

*Ceutorhynchus neglectus* Blatch.

At *Polygonum amphibium* sociés, 2.5 mi. south of Ames, Aug. 9, 1926, one specimen.

*Rhinoncus pericarpus* Fab.

At *Polygonum amphibium* sociés. Earliest adult, Mar. 25, 1928; latest adult, Aug. 7, 1928. Numerous.

*Rhinoncus pyrrhopus* Boh.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, Aug. 17, 1926, and at *Polygonum amphibium* sociés, 2 mi. west of Pacific Junction, July 31, 1928. Two specimens.



*Amalus haemorrhous* Hbst.

At *Spartina* consocies, 8 mi. southeast of Britt, May 19, 1928, one specimen. At *Andropogon furcatus* consocies, 5 mi. east of Renwick, May 19, 1928, one specimen.

*Conotrachelus geminatus* Dej.

Swept from *Ambrosia trifida*, 1 mi. south of Amana, Aug. 12, 1927, one specimen.

*Conotrachelus anaglypticus* Say

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, and 7.75 mi. northwest of Thompson, June 30, 1928. Two specimens.

*Sphenophorus striatipennis* Chttm.

At *Carex* sociies, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Sphenophorus parvulus* Gyll.

At *Spartina* consocies, 2.5 mi. south of Ames, May 21, 1927, one specimen. At *Spartina* consocies, Gitchie-Manito State Park, July 24, 1928, two specimens.

## ORDER TRICHOPTERA

The specimen was determined by Dr. Cornelius Betten.

*Leptocerus* sp.

At *Carex* sociies, Lake Amana, June 23, 1928, one specimen.

## ORDER LEPIDOPTERA

The species were determined by Dr. W. T. M. Forbes, and Messrs. Carl Heinrich, August Busck and W. Schaus. The arrangement follows Barnes and McDunnough (1917).

*Eurymus eriphyle* Edw.

At *Andropogon furcatus* consocies, July 29, 1925, and Aug. 9, 1926. Two specimens.

*Cercyonis alope olympus* Edw.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, Aug. 19, 1925, one specimen. A larva was taken at *Stipa spartea*—*Andropogon scoparius* association northeast of Iowa State College grounds, July 11, 1928. It fed a little on *Andropogon scoparius* leaves, and pupated July 17. The adult appeared Aug. 13, 1928. Several adults were seen at all communities higher than *Spartina* consocies nearly every time they were visited in July and August of each year.

*Chrysophanus hypophlaeas* Bdv.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, June 1, 1926, one specimen.

*Lycaena camyntas* Godt.

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, July 20, 1925, one specimen.

*Celerio lineata* Fabr.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Sept. 13, 1926, one specimen.

*Hyphantria cunea* Dru.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 17, 1926, one specimen.

*Estigmene acrea* Dru.

A larva was taken on *Polygonum amphibium*, 1 mi. south of Ames, Aug. 21, 1928. It fed on *P. amphibium* leaves, and pupated Aug. 25. The adult appeared Sept. 15, 1928. A second larva was taken on *P. amphabium*, 2.5 mi. south of Ames, and the adult appeared Sept. 14, 1928.

*Isia isabella* Sm. and Abb.

A larva of this species was taken at *Andropogon furcatus* consocieties, under a board, Mar. 23, 1926.

*Lygranthoecia brevis* Grote

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 11, 1928, one specimen.

*Lygranthoecia marginata* Haw.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 16, 1926, one specimen, and at *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 17, 1926, one specimen.

*Schinia lynx* Gn.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 1, 1926, one specimen.

*Ceramica picta* Harr.

At *Polygonum amphibium* societies, *Carex* societies, and *Spartina* consocieties, 2.5 mi. south of Ames, the larvae of this species were numerous, June 26, 1926.

*Cirphis unipuncta* Haw.

Three larvae of this species were taken under debris at *Spartina* consocieties, 2.5 mi. north of Ames, May 7, 1928. An adult moth was reared from one of the larvae.

*Cirphis* sp.

One larva was taken at *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Oct. 11, 1926. One larva was taken at *Andropogon furcatus* consocieties, 5 mi. northwest of Buffalo Center, Sept. 16, 1928.

*Nephelodes albilinea* Hbn.

At *Stipa spartea*—*Andropogon furcatus* association, 2 mi. north of Ames, Oct. 11, 1926, seven larvae. At *Andropogon furcatus* consocieties, 5 mi. northwest of Buffalo Center, Sept. 16, 1928, the larvae were numerous on the grass.

*Macronoctua onusta* Grt.

Reared from a larva that was a stalk-borer of *Iris versicolor* which occurred 3.5 mi. north of Ledyard. It was brought in July 23, 1928, pupated Sept. 10, and the adult appeared Sept. 20, 1928.

*Luperina stipata* Morr.

Three larvae were found among roots of *Spartina Michauxiana*, 2.5 mi. north of Ames, July 6, 1928. At *Spartina consocias*, 1 mi. south of Gruver, July 8, 1928, one hundred and eighty larvae were taken from six square feet of sod dug to a depth of nine inches.

*Papaipema marginidens* Gn.

Several larvae as stalk-borers of *Cicuta maculata* which occurred 7.75 mi. northwest of Thompson, were found Aug. 6, 1928.

*Papaipema arctivorens* Hamp.

A larva was taken as a stalk-borer of *Cirsium* sp., 3.5 mi. north of Ledyard, July 7, 1928. It pupated, and the adult appeared Aug. 20, 1928.

*Papaipema cataphracta* Grt.

The larva was taken as a stalk-borer of *Cirsium iowense*, 5 mi. south of Stanhope, July 30, 1928. It pupated Aug. 8, and the moth appeared Sept. 9, 1928.

*Papaipema sciata* Bird

Three larvae, stalk-borers of *Veronica virginica*, were taken 1 mi. south of Amana, July 13, 1928. Two specimens taken from *V. virginica*, 2.5 mi. south of Ames, July 15, 1928.

*Papaipema necopina* Grt.

A larva was taken as a stalk-borer of *Helianthus grosseserratus*, 2.5 mi. south of Ames, Aug. 17, 1928. It pupated, and the moth appeared Sept. 10, 1928.

*Papaipema eryngii* Bird

Five larvae were taken as stalk-borers of *Eryngium yuccifolium*, 5 mi. northwest of Buffalo Center, Aug. 6, 1928. Several pupated about Sept. 5, and an adult appeared Sept. 22, 1928.

*Lithacodia bellicula* Hbn.

At *Andropogon furcatus* consocias, 2.5 mi. south of Ames, July 29, 1925, and 5 mi. east of Renwick, May 19, 1928. One specimen on each date.

*Tarachidia candefacta* Hbn.

At flowers of *Zizia aurea*, 2.5 mi. south of Ames, May 27, 1926. Several specimens were seen, and one taken.

*Caenurgia crassiuscula* Haw.

At *Andropogon furcatus* consocias, 2.5 mi. south of Ames, Aug. 9, 1926, two specimens. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 18, 1927, and July 11, 1928, two specimens.

*Autographa falcifera* Kby.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 3, 1926, one specimen.

*Drasteria erectea* Cram.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 27, 1926, two specimens.

*Ptychopoda inductata* Guen.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 4, 1927, one specimen.

*Eupithecia miserulata* Grote

Two larvae were on leaves of *Zizia aurea*, 1 mi. south of Amana, June 23, 1928. They curled the edges of the leaves, and hid in the folds. The larva pupated in folds of leaves, June 26, and the two adults appeared July 7, 1928.

*Itame flavicaria* Pack.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 19, 1925, two specimens.

*Hesperia tessellata* Scudd.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Sept. 13, 24, 1926, two specimens.

*Nomophila noctuella* D. & S.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 4, 1927, one specimen.

*Pyrausta flavidalis* Guen.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 11, 1928, one specimen.

*Pyrausta futilalis* Led.

Eight larvae were taken at a family of *Apocynum androsaemifolium*, 2.5 mi. south of Ames, July 11, 1928. These larvae with a number of others had spread a web over the upper leaves and stalks of the plants, and were feeding on the leaves. They pupated July 19, 20, 22, and six adults appeared Aug. 8, 10, 11, 1928.

*Pyrausta* sp. near *onythesalis* Wlk.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 11, 1928, one specimen.

*Crambus* sp.

At *Andropogon scoparius*, 7.75 mi. northwest of Thompson, June 30, 1928, numerous. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 4, 1927, one specimen taken and several seen. This species was not represented in the national collection, according to W. Schaus, the determiner.



*Crambus elegans* Cl.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, Aug. 14, 1927, five specimens.

*Tetralopha dolorosella* B. & B.

Two larvae were taken from *Amorpha canescens*, 2 mi. north of Ames. They pupated, and appeared as adults, Sept. 11, 1926. A third moth was reared from leaves of *Psoralea tenuiflora*, and the adult appeared Sept. 8, 1928. The dark green larvae of this species were common on *Amorpha canescens* and *Psoralea tenuiflora*.

*Meroptera pravella* Grote

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, July 29, 1925, one specimen.

*Elasmopalpus lignosellus* Feld.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, Aug. 4, 1927, one specimen.

*Polites cernes* B. & L.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Sept. 13, 1926, one specimen.

*Aegeria* sp.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. north-west of Thompson, Aug. 6, 1928, one specimen.

*Olethreutes hebesana* Wlk.

The work of a stalk-borer was observed on *Pedicularis canadensis*, 2.5 mi. south of Ames, May 12, 1926. It was brought in June 6, and pupated June 8. The adult appeared June 16, 1926.

*Olethreutes coruscana* Clem.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, June 1, 1926, one specimen.

*Sparganothis unifasciana* Clem.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, July 10, 1925, and July 11, 1928, two specimens.

*Sparganothis sulfureana* Clem.

An adult, which appeared Sept. 10, 1927, was reared from a larva that fed upon leaves of *Aster* spp. The larva was taken on *Aster* sp., 2.5 mi. south of Ames. A second moth reared from a larva which fed in the flower of *Lilium philadelphicum* that occurred 1 mi. south of Amana. The adult appeared Aug. 8, 1928.

*Sparganothis pallorana* Rob.

Reared from larva taken on *Amorpha canescens*, 5 mi. south of Stanhope. It pupated June 2, 1928, and the adult appeared June 13, 1928. A second adult was reared from a larva on *A. canescens* that occurred 1 mi. south of Amana, and appeared Aug. 12, 1928. A third adult, dated Sept.

12, 1928, came from a larva taken on *Solidago canadensis*, 1 mi. east of Verdi.

*Apantesis phalerata* Harris

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, Aug. 24, 1928, one specimen.

ORDER DIPTERA

The species were determined by Prof. James S. Hine, Messrs. H. W. Allen, Charles T. Greene, G. S. Walley, and Drs. J. M. Aldrich, Charles P. Alexander, C. Howard Curran, C. L. Fluke, David G. Hall, H. C. Hockett, O. A. Johannssen, and Robert Matheson. The arrangement follows Aldrich (1905).

*Limonia liberta* O. S.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, May 12, 1927; latest adult, Aug. 9, 1928. Not numerous.

*Limonia longipennis* Sch.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, May 12, 1927; latest adult, Aug. 14, 1926. Not numerous.

*Helobia hybrida* Meig.

At all communities except *Bouteloua hirsuta*—*B. curtispindula* and *Andropogon scoparius*—*Bouteloua curtispindula* associations. Earliest adult, Mar. 18, 1927; latest adult, Oct. 20, 1928. Numerous at *Spartina* consocieties.

*Nephrotoma altissima* O. S.

At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, Aug. 9, 1928, one specimen.

*Nephrotoma ferruginea* Fabr.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, chiefly. Earliest adult, May 21, 1927; latest adult, Sept. 19, 1928, at *Stipa spartea*—*Andropogon scoparius* association. Not common.

*Tipula bicornis* Fabr.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, May 30, 1926, June 5, 1927. Two specimens.

*Chironomus barbipes* Staeg.

At *Spartina* consocieties, 8 mi. southeast of Britt, May 19, 1928, two specimens.

*Chironomus brachialis* Coq.

At *Spartina* consocieties, Lake Amana, June 23, 1928, one specimen.

*Chironomus crassicaudata* Mall.

At *Spartina* consocieties and *Carex* societies, chiefly. Earliest adult, May 9, 1926; latest adult, July 23, 1928. Numerous at Lake Amana, June 23, 1928.

*Chironomus decorus* (?) Joh.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 3 mi. south of Muscatine, Sept. 1, 1928, one specimen.

*Chironomus lobiferus* Say

At *Spartina* consocieties, chiefly. Earliest adult, May 12, 1927; latest adult, July 24, 1928. Numerous at Lake Amana, June 23, 1928.

*Chironomus pseudoviridis* Mall.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 3 mi. south of Muscatine, Sept. 1, 1928, one specimen.

*Chironomus tentans* Fab.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, May 12, 1927; latest adult, June 23, 1928. Not common.

*Cricotopus trifascatus* Panz.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 12-21, 1927, five specimens.

*Cricotopus trifasciatus* var. *tricinctus* Meig.

At *Spartina* consocieties, chiefly. Earliest adult, Apr. 16, 1927; latest adult, June 23, 1928. Numerous.

*Cricotopus exilis* Joh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, May 12, 1927, three specimens.

*Cricotopus sylvestris* Fabr.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 20, 1927, one specimen.

*Tanytarsus nigripalpus* Joh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Apr. 16, 1927; latest adult, June 5, 1927. Numerous 2.5 mi. south of Ames.

*Forcipomyia specularis* Coq.

At *Spartina* consocieties, 2.5 mi. south of Ames, Aug. 24, 1928, two specimens, and 5 mi. east of Renwick, Aug. 9, 1928, two specimens. Swept from *Senecio aureus* family, 1 mi. south of Amana, June 23, 1928, one specimen.

*Probezzia* sp. near *opaca* Loew

At *Andropogon furcatus* consocieties, 5 mi. east of Renwick, May 19, 1928, two specimens.

*Protenthes bellus* Loew

Swept from *Rosa pratincola* and *Zizia aurea* families, 1 mi. south of Amana, June 23, 1928, two specimens. At *Carex* societies, same locality and date, one specimen.

*Protenthes stellatus* Coq.

At *Spartina* consocieties and *Carex* sociies, Lake Amana, June 23, 1928, five specimens.

*Tanypus dyari* Coq.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 21, 1927, one specimen.

*Procladius scapularis* Loew

At *Andropogon furcatus*—*Sorghastrum nutans* associies, 3 mi. south of Muscatine, Sept. 1, 1928, one specimen.

*Psorophora ciliata* Fabr.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 26, 1926, one specimen.

*Theobaldia inornata* Will.

At *Andropogon furcatus*—*Spartina Michauxiana* associies, Oct. 9, 1926, Apr. 16, May 21, 1927, three specimens. At *Bouteloua hirsuta*—*B. curtipendula* association, 5 mi. south of Stanhope, Oct. 20, 1928, one specimen.

*Culex pipiens* (?) Linn.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 1925, one specimen.

*Culex tarsalis* Coq.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen.

*Aedes fitchii* F. & Y.

At *Andropogon furcatus* consocieties, 5 mi. northwest of Buffalo Center, Sept. 16, 1928, one specimen.

*Aedes flavescens* Muller

At *Carex* sociies, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Aedes* (?) *intrudens* Dyar

At *Spartina* consocieties, 2.5 mi. south of Ames, July 11, 1928, one specimen.

*Aedes nigromaculis* Ludlow

At *Spartina* consocieties, .5 mi. south of Missouri Valley, Aug. 1, 1928, one specimen.

*Aedes sylvestris* Theob.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 31, 1925, three specimens.

*Aedes triseriatus* Say

At *Andropogon furcatus*—*Sorghastrum nutans* associies, 1 mi. south of Amana, July 20, 1928, two specimens.



*Aedes vexans* Meig.

At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon furcatus*—*Spartina Michauxiana* associes. Earliest adult, June 5, 1927; latest adult, Sept. 15, 1928. Numerous at lights, *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 3, 1926. The most common and numerous mosquito.

*Eugnoriste occidentalis* Coq.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 19, 1926, one specimen.

*Bibio abbreviatus* Loew

At *Andropogon furcatus*—*Spartina Michauxiana* associes. Earliest adult, May 7, 1926; latest adult, May 18, 1928. Numerous at *Andropogon furcatus* consocies, 4 mi. northwest of Thompson, May 18, 1928.

*Bibio longipes* Loew

Swept from *Solidago canadensis*, 7.75 mi. northwest of Thompson, Sept. 15, 1928, two specimens. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Oct. 20, 1928, one specimen.

*Odontomyia pilimanus* Loew

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, two specimens.

*Odontomyia virgo* Wied.

At *Andropogon furcatus* consocies, 6 mi. northwest of Le Mars, July 26, 1928, three specimens.

*Nemotelus carbonarius* Loew

At *Spartina* consocies, 2.5 mi. south of Ames, May 20, 1927, one specimen. Swept from *Solidago* sp., 1.5 mi. northeast of Ocheyedon, July 23, 1928, one specimen.

*Nemotelus unicolor* Loew

At *Spartina* consocies, 2.5 mi. south of Ames, May 20, 1927, two specimens.

*Chrysops sequax* Will.

At *Spartina* consocies, 3.5 mi. north of Ledyard, Aug. 7, 1928, one specimen.

*Tabanus costalis* Wied.

Swept from *Spartina* consocies, chiefly. Earliest adult, July 9, 1928; latest adult, Aug. 13, 1927. Common.

*Tabanus susurrus* Marten

At *Andropogon furcatus* consocies, 2.5 mi. south of Ames, May 31, 1926, one specimen.

*Chrysopila foeda* Loew

At *Spartina* consocies, 2.5 mi. south of Amana, May 21, 1927, two specimens.

*Chrysopila modesta* Loew

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 20, 1925, one specimen.

*Chrysopila proxima* Walk.

At *Carex socias*, Lake Amana, June 23, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, earliest adult, June 30, 1928, and latest adult, Aug. 9, 1928. Not numerous.

*Chrysopila quadrata* Say

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. north-west of Thompson, June 30, 1928, one specimen.

*Exoprosopa fasciata* Macq.

Nearly all specimens were taken at flowers of *Liatris pycnostachya*. Earliest adult, Aug. 5; latest adult, Aug. 18, 1928. Common at *Andropogon furcatus* consocieties.

*Anthrax* sp.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 20, 1926, one specimen.

*Villa fulvohirta* Wied.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 9, 1926, July 10, 1928, three specimens.

*Villa lateralis* Say

At flower of *Steironema lanceolata*, 2.5 mi. south of Ames, Aug. 11, 1928, one specimen.

*Systoechus candidulus* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 5, 1927, three specimens.

*Systoechus vulgaris* Loew

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 20, 1925, one specimen. At flower of *Heliopsis scabra*, same locality, Aug. 26, 1926, one specimen.

*Phthiria punctipennis* Walk.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, one specimen.

*Phthiria sulphurea* Loew

At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedan Mound, July 23, 1928, one specimen, and 2 mi. north of Ames, Aug. 14, 1926, one specimen.

*Sparnopolius fulvus* Wied.

At flowers of *Vernonia* sp. and *Solidago canadensis*, 1.5 mi. east of Muscatine and 1.5 mi. east of Verdi, Sept. 1, 5, 1928. Two specimens.

*Geron senilis* Fabr.

At flower of *Lepachys pinnata*, 2 mi. north of Ames, Aug. 14, 1926, one specimen.

*Psilocephala aldrichii* Coq.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 9, Aug. 9, 1926, two specimens.

*Psilocephala frontalis* Cole

At all communities except *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, May 20, 1927; latest adult, Aug. 19, 1927. Probably common at *Andropogon scoparius*—*Bouteloua curtipendula*, and *Stipa spartea*—*Andropogon scoparius* associations.

*Psilocephala haemorrhoidalis* Macq.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, one specimen. At *Spartina* consocieties, 2.5 mi. north of Ames, Aug. 5, 1927, and 4 mi. north of Le Mars, July 26, 1928, two specimens. At *Andropogon furcatus* consocieties, 6 mi. north of Le Mars, July 26, 1928, one specimen.

*Leptogaster murinus* Loew

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, June 7, 1928; latest adult, Aug. 6, 1928. Common at *Stipa spartea*—*Andropogon scoparius* association.

*Leptogaster flavipes* Loew

At *Carex* societies, 1 mi. south of Amana, June 23, 1928, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manit State Park, July 24, 1928, one specimen.

*Echthodopa pubera* Loew

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 25, 1926, two specimens.

*Holcocephala abdominalis* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10-16, 1925. Common.

*Atomosia puella* Weid.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, one specimen.

*Erax aestuans* Linn.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, 1928, two specimens. At *Spartina* consocieties, 7.75 mi. northwest of Buffalo Center, July 7, 1928, one specimen.

*Promachus vertebratus* Say

At *Andropogon furcatus* consocieties, *Andropogon furcatus*—*Sorghastrum nutans* associates, and *Stipa spartea*—*Andropogon scoparius* association where *Compositae* are more numerous. Earliest adult, Aug. 4, 1928; latest adult, Sept. 19, 1928. Common.

*Asilus erythrocnemius* Hine

At *Andropogon furcatus* consocieties, and *Andropogon scoparius*—*Bouteloua curtipendula* association, chiefly. Earliest adult, June 1, 1926; latest adult, Aug. 9, 1928. Common at *Andropogon furcatus* consocieties.

*Asilus leucopogon* Will.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1928, one specimen.

*Asilus paropus* Walk.

At *Andropogon furcatus* consocieties, and *Stipa spartea*—*Bouteloua curtipendula* association. Earliest adult, July 7, 1928; latest adult, Aug. 6, 1928. Common at *Andropogon furcatus* consocieties.

*Asilus snowii* Hine

At *Spartina* consocieties, 2.5 mi. south of Ames, June 28, 1928, 2 mi. west of Kelso, July 30, 1928, and 5 mi. northwest of Buffalo Center, July 7, 1928. At *Andropogon scoparius*, 7.75 mi. northwest of Thompson, Aug. 6, 1928. One specimen on each date.

*Psilopodinus sipho* Say

At all communities except *Andropogon furcatus*—*Sorghastrum nutans* associes, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, July 16, 1925; latest adult, Aug. 26, 1926. Common at *Spartina* consocieties.

*Sciapus caudatus* Weid.

At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, Aug. 7, 1928, and 5 mi. east of Renwick, Aug. 9, 1928, two specimens.

*Sciapus flavipes* Ald.

At *Spartina* consocieties. Earliest adult, Aug. 1, 1928; latest adult, Aug. 9, 1928. Numerous.

*Chrysotus hirtipes* V. D.

At *Carex* socies, Lake Amana, June 23, 1928, one specimen.

*Chrysotus picticornis* Loew

At *Spartina* consocieties, 2.5 mi. south of Ames, May 20, 30, 1927, four specimens.

*Medeterus lobatus* V. D.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen. At *Carex* socies, same locality, Aug. 6, 1928, one specimen.

*Medeterus veles* Loew

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg, July 30, 1928, one specimen.

*Dolichopus bifractus* Loew

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, July 22, 1925; latest adult, Sept. 15, 1927. Common.



*Dolichopus comatus* Loew

At *Andropogon furcatus*—*Spartina Michauxiana* associates, chiefly. Earliest adult, May 12, 1927; latest adult, Aug. 4, 1927. Common at *Spartina* consocieties.

*Dolichopus latipes* Loew

At *Spartina* consocieties, and *Carex* societies. Earliest adult, June 30, 1928; latest adult, Aug. 12, 1927. Scarce.

*Dolichopus pachynemus* Loew

At *Spartina* consocieties, Lake Amana, June 23, 1928, two specimens.

*Dolichopus ramifer* Loew

At *Spartina* consocieties, chiefly. Earliest adult, May 2, 1928; latest adult, July 30, 1928. Common.

*Pelastoneurus vagans* Loew

At *Spartina* consocieties, chiefly. Earliest adult, May 12, 1928; latest adult, Aug. 1, 1928. Common.

*Drapetis unipila* Loew

At *Spartina* consocieties, 4 mi. northwest of Thompson, May 18, 1928, one specimen.

*Platypalpus holosericeus* Mel.

At *Polygonum amphibium* societies, 6 mi. northwest of Ledyard, Aug. 7, 1928, one specimen.

*Hybos triplex* Walk.

At *Spartina* consocieties, and *Carex* societies, Lake Amana, June 23, 1928, two specimens. At *Andropogon scoparius*, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Empis nuda* Loew

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 8 mi. southeast of Britt and 7.75 mi. northwest of Thompson, May 18, 1928. Numerous.

*Pipunculus affinis* Cres.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 26, 1926, one specimen. At *Polygonum amphibium* societies, 10 mi. southwest of Kelso, July 30, 1928, one specimen.

*Pipunculus fasciatus* Loew

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 16, 1925, one specimen.

*Pipizella pulchella* Will.

At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen.

*Paragus bicolor* Fabr.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 12, 1927, and 7.75 mi. northwest of Thompson, Aug. 6, 1928. Two specimens.

*Paragus tibialis* Fall.

At *Andropogon furcatus* consociés, 3.5 mi. north of Ledyard, July 7, 1928, one specimen. At *Stipa spartea*—*Bouteloua curtipendula* association, 5 mi. south of Stanhope, Aug. 5, 1927, four specimens.

*Platychirus immarginatus* Zett.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, chiefly. Earliest adult, May 12, 1928; latest adult, Sept. 19, 1928. Common.

*Platychirus quadratus* Say

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, May 12, 1927; latest adult, Sept. 19, 1928. Not common.

*Melanostoma mellinum* Linn.

At *Spartina* consociés, 8 mi. southeast of Britt, May 19, 1928, and 6 mi. northwest of Ledyard, Aug. 7, 1928. Two specimens.

*Allograpta obliqua* Say

Nearly all specimens were taken at flowers of *Silphium laciniatum*, 2.5 mi. south of Ames, July 27, 1926, where they were numerous. At *Rosa pratincola* flower, 8 mi. southeast of Britt, July 6, 1928, one specimen.

*Mesogramma marginata* Say

At all communities. Earliest adult, June 5, 1927; latest adult, Sept. 15, 1928. The most common syrphid. Numerous at flowers of *Cicuta maculata*, *Rosa pratincola*, and *Rudbeckia hirta*.

*Mesogramma polita* Say

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 3 mi. south of Muscatine, Sept. 1, 1928, four specimens, 1.5 mi. east of Verdi, Aug. 20, Sept. 5, 1927, two specimens.

*Sphaerophoria cylindrica* Say

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association. Numerous at flowers of *Cicuta maculata* and *Rudbeckia hirta*. Common, but not as numerous as *Mesogramma marginata*.

*Sphaerophoria scripta* Linn.

At flowers of *Cicuta maculata*, 2.5 mi. south of Ames, July 10, 1928. At flower of *Rudbeckia hirta*, 8 mi. southeast of Britt, July 6, 1928. At flowers of *Cicuta maculata*, 5 mi. east of Renwick, July 8, 1928. At *Bouteloua hirsuta*—*B. curtipendula* association, 5 mi. south of Stanhope, Sept. 19, 1928. One specimen at each locality.

*Neoascia globosa* Walk.

At *Spartina* consociés, 4 mi. northwest of Thompson, May 18, 1928, one specimen.

*Eristalis latifrons* Loew

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, Aug. 11, 1925. At flower of *Liatris pycnostachya*, same locality, Aug. 11, 1927, and

at flower of *Rudbeckia hirta*, same locality, July 9, 1928. One specimen on each date.

*Helophilus latifrons* Loew

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, Aug. 11, 1925, one specimen. At flowers of *Cassia Chamaecrista*, Aug. 12, 1927, one specimen.

*Syritta pipiens* Linn.

At flowers of *Cicuta maculata*, 2.5 mi. south of Ames, Aug. 4, 1928, one specimen.

*Zodion fulvifrons* Say

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, July 10, 1925, one specimen.

*Zodion obliquefasciatum* Macq.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, July 2, 1926, four specimens. At flower of *Helianthus* sp., same locality, Aug. 9, 1926, one specimen.

*Oncomyia abbreviata* Loew

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, two specimens. At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedon Mound, July 23, 1928, one specimen.

*Phorantha occidentis* Walk.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 17, 1926, one specimen.

*Schizotachina convecta* Walk.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 11, 1925, one specimen.

*Celatoria diabroticae* Shim.

At *Bouteloua hirsuta*—*B. curtipendula* association, 5 mi. south of Stanhope, Sept. 19, 1928, one specimen.

*Polidea areos* Walk.

At *Spartina* consociés, 2.5 mi. south of Ames, Aug. 9, 1926, two specimens. At *Polygonum amphibium* sociés, same locality and date, one specimen. At flowers of *Liatris pycnostachya*, same locality, July 30, 1926, one specimen.

*Leucostoma atra* Tns.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, and Sergeant Bluff, July 26, 1928. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 5, 1927. One specimen on each date.

*Siphophyto floridensis* Tns.

At *Andropogon furcatus* consociés, 3.5 mi. north of Ledyard, July 7, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, Aug. 6, 1928, one specimen.

*Phytoadmontia setigera* Coq.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Sept. 26, 1926, one specimen.

*Cylindromyia argentea* Tns.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 18, 1926, one specimen.

*Cylindromyia decora* Aldrich

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 19, 1926, one specimen, and 3.5 mi. north of Ledyard, July 7, 1928, two specimens. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 3 mi. north of Muscatine, Sept. 1, 1928, one specimen.

*Voria ruralis* Meig.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 25, 1926, one specimen.

*Ernestia ampelus* Walk.

At *Andropogon furcatus* consocieties, 5 mi. east of Renwick, May 9, 1928, one specimen.

*Exorista nigripalpis* Tns.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, June 26-July 6, 1926. Most of the specimens were swept from flowers of *Cicuta maculata*. Common at *Spartina* consocieties.

*Exorista simulans* Meig.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen. At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen.

*Phorocera claripennis* Macq.

Swept from flowers of *Cicuta maculata*, 2.5 mi. south of Ames, July 6, 1926, two specimens.

*Doryphorophaga doryphorae* Riley

At *Spartina* consocieties, 5 mi. east of Renwick, Aug. 9, 1928, one specimen.

*Zenillea crassiseta* A. & W.

At flower of *Rudbeckia hirta*, 5 mi. east of Renwick, July 7, 1928, one specimen. At flowers of *Cicuta maculata*, same locality and date, July 8, 1928, one specimen.

*Masicera pauciseta* Coq.

At flowers of *Vernonia noveboracensis*, 2.5 mi. south of Ames, July 19, 1928, two specimens.

*Masicera* near *rutila* Meig.

At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, July 7, 1928, one specimen.



*Erycia myoidaea* Desv.

Swept from flowers of *Cicuta maculata*, 2.5 mi. south of Ames, Aug. 4, 1928, one specimen. At *Andropogon scoparius*, 7.75 mi. northwest of Thompson, Aug. 6, 1928, one specimen.

*Ceromasia senilis* Meig.

At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, July 7, 1928, one specimen.

*Tachinomyia robusta* Town.

At *Spartina* consocieties, 5 mi. east of Renwick, May 8, 1926, one specimen.

*Winthemia quadripustulata* Fabr.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 9, 1926, one specimen. At *Andropogon furcatus*—*Sorghastrum nutans* association, 6 mi. south of Washington, Sept. 7, 1927, one specimen. Two specimens were reared as parasites of a salt-marsh caterpillar (*Estigmene acrea* Dru.) taken on *Polygonum amphibium*, 2.5 mi. south of Ames, July 11, 1928. The maggots left the host and pupated July 19, 1928. The adult flies appeared Sept. 21, 1928.

*Metachaeta atra* Coq.

At flower of *Rudbeckia hirta*, 1 mi. south of Amana, June 23, 1928. At *Stipa spartea*—*Bouteloua curtipendula* association, 7.75 mi. northwest of Thompson, May 18, 1928. One specimen was reared from an armyworm (*Cirphis unipuncta* Haw.). The host was taken on *Andropogon furcatus*, 1.5 mi. east of Verdi, Sept. 5, 1928. The maggot pupated outside of the host, and the adult fly appeared Oct. 8, 1928.

*Brachycoma apicalis* Coq.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, one specimen.

*Gonia sequax* Will.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Oct. 1, 1926, one specimen.

*Gonia turgida* Coq.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 16, 1926, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Apr. 25, Sept. 19, 1928, three specimens.

*Microphthalma disjuncta* Wied.

At *Spartina* consocieties, 4 mi. northwest of Le Mars, July 26, 1928, one specimen.

*Microphthalma michiganensis* Town.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 3, 1926. The specimen came to lights at night.

*Myiocera cremides* Walk.

At flowers of *Rudbeckia hirta*, 8 mi. southeast of Britt, July 6, 1928, one specimen. At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, July 7, 1928, two specimens. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, two specimens.

*Ptilodexia tibialis* Desv.

At flowers of *Rudbeckia hirta*, 8 mi. east of Britt, July 6, 1928, one specimen. At *Andropogon furcatus* consocieties and *Stipa spartea*—*Andropogon scoparius* association, 6 mi. northwest of Ledyard, Aug. 7, 1928, two specimens. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Sarcophaga communis* Park.

At *Spartina* consocieties, Lake Amana, Aug. 12, 1927, and June 23, 1928, two specimens. At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen.

*Sarcophaga heliciis* Tns.

At flower of *Rudbeckia hirta*, 2 mi. north of Ames, Aug. 25, 1926, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 4, 1925, one specimen, and 8 mi. southeast of Britt, May 19, 1928, two specimens. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 mi. south of Washington, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, May 9, 1928, one specimen, and 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Sarcophaga hunteri* Hg.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 21, 1925, Sept. 24, 1926, two specimens. At *Andropogon furcatus* consocieties, 5 mi. northwest of Buffalo Center, Sept. 16, 1928, one specimen. At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, two specimens.

*Sarcophaga latisetosa* Park.

At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 5, 1928, one specimen. At *Andropogon furcatus* consocieties, 4 mi. northwest of Thompson, May 18, 1928, one specimen. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1.5 mi. east of Verdi, Aug. 20, 1928, one specimen.

*Sarcophaga pachyprocta* Park.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 13, 1925, Sept. 24, 1926, three specimens. At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, Aug. 6, 1928, one specimen.

*Sarcophaga pallinervis* Thom.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 8, 1925, and 7.75 mi. northwest of Thompson, June 30, 1928;

one specimen at each locality. At flowers of *Solidago* sp., 2 mi. north of Ames, Sept. 24, 1926, three specimens. At *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927, one specimen. At *Andropogon furcatus* consociates, 8 mi. southeast of Britt, July 6, 1928, and 5 mi. east of Renwick, May 8, 1926; one specimen at each locality.

*Sarcophaga quadrisetosa* Coq.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, Aug. 6, 1928, one specimen.

*Sarcophaga sinuata* Mg.

At flowers of *Solidago* spp., 2 miles north of Ames, Sept. 24, 1926, and 6 mi. south of Washington, Aug. 24, 1928; one specimen at each locality. At *Spartina* consociates, 2.5 mi. north of Ames, Aug. 5, 1927, two specimens, and Lake Amana, June 23, 1928, one specimen. At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen

*Sarcophaga spatulata* Ald.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 5 mi. northwest of Buffalo Center, July 7, Sept. 6, 1928, two specimens.

*Opelousia obscura* Tns.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, June 30, Aug. 6, 1928, two specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, one specimen. At *Polygonum amphibium* sociates, .5 mi. south of Missouri Valley, Aug. 1, 1928, one specimen.

*Lucilia caesar* Linn.

At *Spartina* consociates, 2.5 mi. south of Ames, May 20, 1927, one specimen.

*Morellia micans* Macq.

Swept from flowers of *Cicuta maculata*, 2.5 mi. south of Ames, Aug. 4, 1928, one specimen.

*Musca domestica* Linn.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Stomoxys calcitrans* Linn.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 5, 1927, one specimen.

*Phaonia deleta* Stein

At *Spartina* consociates, Lake Amana, June 23, 1928, one female specimen.

*Limnophora narona* Walk.

At *Spartina* consociates, chiefly. Earliest adult, June 5, 1927; latest adult, Aug. 19, 1927. Not numerous.

*Calythea pratincta* Pnz.

At *Spartina* consocieties, 7.75 mi. northwest of Thompson, Aug. 6, 1928, one specimen.

*Acroptena ambigua* Fall.

At *Spartina* consocieties, Lake Amana, June 23, 1928, one specimen.

*Hydrophoria divisa* Meig.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 17, 1927, one specimen.

*Hylemyia antiqua* Meig.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, May 18, 1928, one specimen.

*Hylemyia cilicrura* Rond.

At all communities except *Bouteloua hirsuta*—*B. curtipendula* and *Andropogon scoparius*—*Bouteloua curtipendula* associations. Earliest adult, May 9, 1928; latest adult, Oct. 20, 1928. Numerous at *Stipa spartea*—*Andropogon scoparius* association.

*Hylemyia depressa* Stein

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 4, 1927, one specimen.

*Hylemyia fugax* Meig.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, May 18, 1928, one specimen.

*Hylemyia parva* R. D.

At all communities except *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, June 23, 1928; latest adult, Aug. 6, 1928. Scarce.

*Hylemyia testacea* Stein

Swept from flowers of *Solidago canadensis*, 1 mi. south of Amana, two specimens, male and female.

*Hylemyia variata* Fall.

At *Spartina* consocieties, 1 mi. south of Amana and Lake Amana, June 23, 1928, two specimens. At *Carex* societies, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen.

*Hylemyia salicola* Huck.

At *Spartina* consocieties, 2.5 mi. north of Ames, May 7, 1928, one female specimen.

*Eremomyia apicalis* (?) Stein

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 18, 25, 1927, four specimens. At *Andropogon furcatus* consocieties, 2 mi. south of Ledyard, May 9, 1926, one specimen. At flowers of *Solidago canadensis*, 1 mi. south of Amana, Aug. 25, 1927, one specimen.



*Hammomyia unilineata* Zett.

At *Spartina* consocieties, 2.5 mi. north of Ames, May 2, 1928, one specimen.

*Paregle cinerea* Fall.

At *Spartina* consocieties, 1 mi. south of Amana, June 23, 1928, one specimen, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen, and 7.75 mi. northwest of Thompson, Aug. 6, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, May 18, 1928, one specimen. At *Andropogon furcatus* consocieties, 2 mi. south of Ledyard, May 9, 1926, one specimen.

*Paregle cinerella* Fall.

At flower of *Anemone patens* var. *Wolfgangiana*, 2 mi. north of Ames, Apr. 9, 1928, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen. At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928, two specimens.

*Pegomyia hyoscyami* Panz.

Swept from *Helianthus occidentale*, 5 mi. south of Stanhope, Aug. 19, 1927, one specimen.

*Pegomyia luteola* Mall.

Swept from *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927, one specimen. At *Spartina* consocieties, same locality, June 23, 1928, two specimens.

*Pentacricia aldrichii* Stein

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 13, 1926, one female specimen.

*Coenosia alticola* (?) Mall.

At *Spartina* consocieties, 2.5 mi. north of Ames, May 2, 1928, one specimen.

*Coenosia denticornis* Mall.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 16, 20, 1925, three specimens.

*Coenosia hypopygialis* Stein

At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, Aug. 9, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, Aug. 6, 1928, one specimen.

*Coenosia lata* Walk.

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, May 2, 1928; latest adult, Sept. 15, 1928. Numerous.

*Schoenomyza dorsalis* Loew

At *Andropogon furcatus*—*Spartina Michauxiana* associates, chiefly. Earliest adult, Mar. 25, 1928; latest adult, Aug. 9, 1928. Numerous during April and May.

*Schoenomyza litorella* Fall.

At *Spartina* consocieties, 2.5 mi. south of Ames, Apr. 22, 1928, one specimen.

*Lispa uliginosa* Fall.

At flowers of *Rudbeckia hirta*, 1 mi. south of Amana, June 23, 1928, one specimen.

*Parallelomma varipes* Walk.

At *Polygonum amphibium* sociies, 2.5 mi. south of Ames, July 26, Aug. 2, 1926, two specimens. At *Spartina* consocieties, 2.5 mi. north of Ames, Aug. 5, 1927, and Lake Amana, June 23, 1928, two specimens.

*Scatophaga furcata* Say

At *Spartina* consocieties, 2.5 mi. south of Ames. Earliest adult, Mar. 25, 1928; latest adult, May 30, 1928. Not numerous.

*Leptocera carinata* Spul.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen.

*Leptocera fontinalis* Fall.

At lights, *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 3, 1926, numerous. At *Spartina* consocieties, 2.5 mi. south of Ames, May 12, June 5, 1927, numerous. At *Anemone canadense* flowers, 1 mi. south of Amana, June 23, 1928, one specimen.

*Leptocera lutosa* Stenh.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 12, June 5, 1927, and Mar. 25, 1928. Four specimens.

*Borborus equinus* Fall.

At *Stipa spartea*—*Bouteloua curtipendula* association, 2 mi. north of Ames, Apr. 25, 1927, one specimen. At *Bouteloua hirsuta*—*B. curtipendula* association, 5 mi. south of Stanhope, Oct. 20, 1928, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Mar. 18, May 12, 1927, two specimens. At *Spartina* consocieties, 2.5 mi. south of Ames, May 20, 1927, one specimen.

*Scatophora carolinensis* Desv.

At *Spartina* consocieties, 2.5 mi. south of Ames, Mar. 25, 1928, three specimens.

*Melina nana* Fall.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 14, Oct. 1, 1926, two specimens. At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen, and 8 mi. southeast of Britt, May 19, 1928, three specimens.

*Melina obtusa* Fall.

At *Carex* sociies 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Tetanocera elata* Fabr.

At *Spartina* consocieties, chiefly. Earliest adult, May 12, 1927; latest adult, Sept. 16, 1928. Common.

*Dictya umbrarum* Linn.

At all communities except *Andropogon scoparius*—*Bouteloua curtipendula*, and *Bouteloua hirsuta*—*B. curtipendula* associations. Earliest adult, Mar. 25, 1928; latest adult, Sept. 7, 1928. Numerous at *Spartina* consocieties.

*Limnia saratogensis* Fitch

At all communities. Earliest adult, June 15, 1928; latest adult, Oct. 20, 1928. Common at all communities except *Bouteloua hirsuta*—*B. curtipendula* and *Andropogon scoparius*—*Bouteloua curtipendula* associations.

*Minettia lupulina* Fabr.

Swept from *Senecio aureus*, 1 mi. south of Amana, June 23, 1928, one specimen.

*Camptoprosopella vulgaris* Fitch

At flowers of *Erigeron ramosus*, 2 mi. north of Ames, June 25, 1925, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, June 15, 1928, one specimen.

*Pyrgota undata* Wied.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1926, one specimen.

*Rivellia flavimana* Loew

At *Andropogon furcatus* consocieties, *Stipa spartea*—*Andropogon furcatus* association, and *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, June 15, 1928; latest adult, Aug. 9, 1928. Not common.

*Rivellia viridulans* Desv.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Andropogon furcatus*—*Sorghastrum nutans* associates, chiefly. Common at *Spartina* consocieties. Earliest adult, May 31, 1926; latest adult, Sept. 1, 1928.

*Myrmecomylia fenestrata* Coq.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 26, Aug. 14, 1926, three specimens. At *Stipa spartea*—*Bouteloua curtipendula* association, 2 mi. north of Ames, Aug. 9, 1926, one specimen.

*Tritoxa flexa* Wied.

At *Andropogon furcatus* consocieties, 3.5 mi. north of Ledyard, July 7, 1928, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, one specimen.

*Tritoxa incurva* Loew

At *Andropogon furcatus* and *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927, three specimens. At *Andropogon furcatus* consocieties, 6 mi. northwest of Cedar Falls, July 17, 1928, two specimens.

*Eumetopiella rufipes* Macq.

At *Spartina* consocieties, 2.5 mi. north of Ames, Aug. 5, 1927, 1 mi. south of Amana, Aug. 13, 1927, and .5 mi. south of Missouri Valley, Aug. 1, 1928. Three specimens.

*Tephronota narytia* Walk.

Swept from *Andropogon furcatus*, 1.5 mi. east of Verdi, Aug. 20, 1928, two specimens.

*Melieria obscuricornis* Loew

Swept from *Phragmites communis* family, 5 mi. northwest of Buffalo Center, Aug. 6, 1926, seven specimens. At *Spartina* consocieties, 2.5 mi. south of Ames, June 26, 1926, three specimens and 4 mi. northwest of Le Mars, July 26, 1928, one specimen.

*Melieria ochricornis* Loew

At *Spartina* consocieties, chiefly. Earliest adult, May 2, 1926; latest adult, Aug. 9, 1928. Common.

*Chrysomyza demandata* Fabr.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Chaetopsis aenea* Wied.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Andropogon furcatus* consocieties, chiefly. Earliest adult, May 12, 1927; latest adult, Aug. 24, 1928. Common at *Spartina* consocieties.

*Stenomyia nasoni* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 18, 1926, one specimen.

*Straussia longipennis* Wied.

Swept from *Helianthus grosseserratus*, Lake Amana, June 25, 1928, one specimen.

*Eurosta comma* Wied.

At *Andropogon furcatus* consocieties, 5 mi. northwest of Buffalo Center, Sept. 16, 1928, one specimen.

*Ensina humilis* Loew

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association, and *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, May 19, 1928; latest adult, Aug. 6, 1928. Common.

*Tephritis aequalis* Loew

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, July 24, 1928; latest adult, Aug. 7, 1928. Scarce at each community.

*Tephritis finalis* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 25, Oct. 1, 1926, three specimens.



*Euaresta festiva* Loew

At *Spartina* consocieties, 2.5 mi. north of Ames, Aug. 5, 1927, and 4 mi. north of Le Mars, July 26, 1928. Two specimens.

*Euaresta bella* Loew

At all communities except *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, July 16, 1926; latest adult, Sept. 15, 1928. Common; numerous at *Andropogon furcatus* consocieties.

*Urellia solaris* Loew

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 20, 1925, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedan Mound, July 23, 1928, one specimen.

*Calobata alesia* Walk.

At *Carex* societies, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen. At *Spartina* consocieties, Lake Amana, June 23, 1928, one specimen.

*Themira putris* Linn.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen.

*Sepsis violacea* Meig.

At *Andropogon furcatus*—*Spartina Michauxiana* societies, *Andropogon furcatus*—*Sorghastrum nutans* societies, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 12, 1927; latest adult, Sept. 20, 1928. Common at *Spartina* consocieties.

*Saltella scutellaris ruficoxa* Macq.

Swept from flowers of *Solidago Riddellii*, 6 mi. south of Washington, Aug. 24, 1926, one specimen.

*Saltella scutellaris* Fall.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 29, 1925, one specimen. At flowers of *Pycnanthemum* sp., 2 mi. north of Ames, Aug. 14, 1926, one specimen.

*Loxocera fumipennis* Coq.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 30, 1927, one specimen.

*Notiphila scalaris* Loew

At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen.

*Paralimna appendiculata* Loew

Swept from *Carex* societies, Lake Amana, June 23, 1928, one specimen.

*Hydrellia scapularis* Loew

At *Spartina* consocieties, 2.5 mi. south of Ames, June 5, 1927, one specimen.

*Philygria debilis* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 9, 1928, 3.5 mi. north of Ledyard, Aug. 7, 1928, and 5 mi. south of Stanhope, Apr. 25, 1928; one specimen at each locality. At *Spartina consocias*, 8 mi. southeast of Britt, May 19, 1928, one specimen.

*Lytogaster grvida* Loew

At *Spartina consocias*, 2.5 mi. south of Ames, June 5, 1927, two specimens.

*Scatella stagnalis* Fall.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 mi. south of Ames, Apr. 24-May 12, 1927. Common.

*Meromyza americana* Fitch

At all communities except *Andropogon scoparius*—*Bouteloua curtipendula* and *Bouteloua hirsuta*—*B. curtipendula* associations. Earliest adult, May 5, 1926; latest adult, Aug. 9, 1928. Numerous at *Andropogon furcatus* consocias.

*Chlorops certima* Adams

At *Spartina consocias*, chiefly. Earliest adult, May 12, 1927; latest adult, Aug. 6, 1928. Scarce.

*Chlorops glabra* Meig.

At *Spartina consocias* and *Carex* socies. Earliest adult, June 23, 1928; latest adult, Sept. 15, 1928. Scarce.

*Chlorops grata* Loew

At *Carex* socies, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Chlorops obscuricornis* Loew

At *Andropogon furcatus*—*Spartina Michauxiana* associates, *Andropogon furcatus*—*Sorghastrum nutans* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 9, 1926; latest adult, Sept. 15, 1928. Not common at any community.

*Epichlorops puncticollis* Zh.

At *Andropogon furcatus* consocias, 2 mi. south of Ledyard, May 9, 1926, one specimen. At *Spartina consocias*, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Diplotoxa microcera* Loew

At *Spartina consocias*, 2.5 mi. south of Ames, May 12, 1927, one specimen.

*Diplotoxa versicolor* Loew

At *Stipa spartea*—*Andropogon* association, chiefly. Earliest adult, May 19, 1928, latest adult, Aug. 7, 1928. Common.

*Ectecephala similis* Becker

At *Stipa spartea*—*Andropogon scoparius* association, chiefly. Earliest adult, June 30, 1928, latest adult, Aug. 7, 1928. Common.

*Crassiseta decipiens* Loew

At *Polygonum amphibium* socris, .5 mi. south of Missouri Valley, Aug. 1, 1928, one specimen.

*Crassiseta nigriceps* Loew

At *Spartina* consociis, Lake Amana, June 23, 1928, one specimen.

*Hippelates stramineus* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 13, 1926, one specimen.

*Madiza cinerea* Loew

Swept from *Senecio aureus* family, 1 mi. south of Amana, June 23, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, Sept. 15, 1928, one specimen.

*Oscinella coxendix* Fitch

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 19, 1926, and 5 mi. northwest of Buffalo Center, July 7, 1928, three specimens. At *Andropogon furcatus* consociis, 2.5 mi. south of Ames, July 22, 1925, one specimen.

*Oscinella criddlei* Ald.

At *Spartina* consociis, 1 mi. south of Amana, June 23, 1928, three specimens.

*Oscinella dissidens* Tuck.

At *Spartina* consociis, 4 mi. northwest of Le Mars, July 26, 1928, one specimen.

*Oscinella dorsata* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 18, 1926, four specimens; 5 mi. south of Stanhope, June 15, 1928, one specimen, and 7.75 mi. northwest of Thompson, May 18, 1928, one specimen. At *Spartina* consociis, Lake Amana, June 23, 1928, one specimen.

*Oscinella frit* Linn.

At flower of *Fragaria virginiana*, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Oscinella salina* Curran

At *Spartina* consociis. Earliest adult, May 18, 1928; latest adult, June 5, 1927. Not common.

*Anthomyza tenuis* Loew

At *Stipa spartea*—*Bouteloua curtipendula* association, 2 mi. north of Ames, June 25, 1925, one specimen.

*Anthomyza variegata* Loew

At *Spartina* consociis, Lake Amana, June 23, 1928, one specimen.

*Napomyza lateralis* Fall.

Reared from flowers of *Pedicularis canadensis*. Pupated May 17, 1926, and adults appeared May 25, 1926. Two specimens.

*Agromyza coquilletti* Mall.

At *Andropogon furcatus* consocieties, Apr. 24, 1927, one specimen.

*Agromyza laterella* Zett.

At *Spartina* consocieties, 4 mi. northwest of Thompson, May 18, 1928, one specimen.

*Agromyza platyptera* Thoms.

At *Spartina* consocieties, Lake Amana, June 23, 1928, one specimen. At *Andropogon furcatus* consocieties, 5 mi. east of Renwick, Aug. 9, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, May 9, 1928, one specimen.

*Agromyza angulata* Loew

At *Spartina* consocieties, Lake Amana, June 23, 1928, one specimen, and 1 mi. south of Amana, June 23, 1928, one specimen.

*Agromyza tilliae* Caud.

Ovipositing in flower bud of *Lepachys pinnata*, 2.5 mi. south of Ames, July 10, 1928, one specimen. At *Andropogon furcatus* consocieties, same locality, July 10, Aug. 4, 1928, two specimens.

*Cerodontha dorsalis* Loew

At *Andropogon furcatus* consocieties, 8 mi. southeast of Britt, Aug. 9, 1928, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Meoneura vagans* Fall.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 18, 1926, one specimen.

*Desmometopa tarsalis* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 5, 1927, one specimen.

*Euchlorops vittata* Mall.

At *Stipa spartea*—*Andropogon scoparius* association, Sept. 17, Oct. 9, 1926, two specimens, 2 mi. north of Ames.

*Milichiella lacteipennis* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen.

*Pholeomyia indecora* Loew

At *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 29, 1925; latest adult, Aug. 13, 1925. Common.



*Pseudodinia polita* Mall.

At *Spartina* consocieties, 1 mi. south of Amana, Aug. 13, 1927, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 26, 1926, one specimen.

*Mallochiella latipes* Meig.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 22, 1928, one specimen.

*Leucopis bella* Loew

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Leucopis griseola* Fall.

At lights, *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 3, 1926, one specimen.

*Chamaemyia elegans* Panz.

At *Carex* societies, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 21, 1925, one specimen.

*Chamaemyia polystigma* Meig.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 25, Sept. 17, 1926, three specimens.

## ORDER HYMENOPTERA

The species were determined by Miss Grace Sandhouse, Messrs. S. A. Rohwer, R. A. Cushman, A. B. Gahan, L. H. Weld, W. M. Mann, and Drs. Herbert H. Ross, Theodore H. Frison, and M. R. Smith. The arrangement follows Britton (1916).

*Empria capillata* Mac G.

At *Andropogon furcatus* consocieties, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, Apr. 16, 1927; latest adult, Apr. 30, 1926. Five specimens.

*Empria contorta* Mac G.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 20, 1926, one specimen. At flower of *Fragaria virginiana*, 2.5 mi. south of Ames, May 5, 1926, one female specimen.

*Dolerus collaris* Say

At *Stipa spartea*—*Andropogon scoparius* association and *Andropogon furcatus* consocieties. Earliest adult, Apr. 21, 1926; latest adult, May 12, 1927. Five specimens.

*Dolerus similis* Nort.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 21, 1926, one specimen.

*Pristiphora bivittata* Nort.

At *Spartina* consocieties, 1 mi. south of Amana, Aug. 13, 1927, one specimen.

*Pachynematus extensicornis* Nort.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 3, 1926, one specimen. At *Andropogon furcatus* consocieties, 5 mi. east of Renwick, May 19, 1928, one specimen.

*Pachynematus affinis* Marl.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 5, 1926, one specimen, and 2 mi. south of Ledyard, May 9, 1926, one specimen.

*Pachynematus auratus* Marl.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 21, 1926, three specimens. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 19, 21, 1926, five specimens.

*Anoplolyda cavifrons* Cr.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 5, 1926, one specimen.

*Paracharactus rudis* Nort.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. north-west of Thompson, May 19, 1928, one specimen.

*Sterictiphora lineata* Roh.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 17, 1926, one specimen.

*Cephus cinctus* Nort.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 23, 1926, one specimen.

*Apanteles bedelliae* Vier.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10, 1925, one specimen.

*Apanteles crassicornis* Prov.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 11, 1925, two specimens.

*Apanteles ensiger* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 29, 1925, two specimens.

*Apanteles harti* Cush.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 7, 1926, one specimen.

*Apanteles plathypenae* Mues.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 12, 1926, one specimen.

*Apanteles trachynotus* Vier.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 23, 1926, one specimen.

*Microgaster auripes* Prov.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 25, 1926, one specimen. At *Andropogon furcatus* consocieties, May 19, 1926, one specimen.

*Microgaster pantographae* Mues.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 19, 1926, one specimen.

*Microplitis brassicae* Mues.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 9, 1926, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 5, 1926, one specimen.

*Microbracon mellitor* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 1925, May 5, 21, 1926. Four specimens.

*Perilitus eleodis* Vier.

At *Polygonum amphibium* societies, 2.5 mi. south of Ames, June 26, Aug. 2, 1926, two specimens.

*Orgilus detectiformis* Vier.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 19, Sept. 17, 1926, two specimens.

*Bassus simillimus* Cress.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 29, 1925, one specimen.

*Bracon nigrosternum* Morr.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 1925. At *Stipa spartea*—*Andropogon scoparius* association, 2.5 mi. south of Ames, Aug. 4, 1926, one specimen.

*Ascogaster provancheri* D. T.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 13, 16, 1926, two specimens.

*Ascogaster carpocapsae* Vier.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 19, 1926, one specimen.

*Ascogaster erythrethorax* Vier.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 18, 1926, one specimen.

*Polystenidea parksi* Vier.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 18, 1926, one specimen.

*Viereckiana bellulus* D. T.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 21, 1926, one specimen.

*Paniscus geminatus* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 17, 1926, one specimen.

*Paniscus ocellatus* Vier.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 21, 1926; latest adult, Aug. 9, 1926. Five specimens.

*Schizoprymnus phillipsi* Vier.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 16, 20, 1925, two specimens.

*Anomalon ejuncidum* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 7, 1926, one specimen.

*Habronyx apicalis* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Sept. 24, 1926, one specimen.

*Paranomalon semirufum* Nort.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 2, 1926, one specimen.

*Paranomalon propinquum* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 12, 1926, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 31, 1926, one specimen.

*Ophion abnormis* Felt

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 12, 1926, one specimen. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 19, 1926, one specimen.

*Ophion bifoveolatum* Brulle

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 17, 1926, one specimen.

*Ophion idoneum* Vier.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Apr. 19, 1926, one specimen.

*Tryphon seminiger* Cress.

At *Spartina* consocieties and *Carex* societies, 2.5 mi. south of Ames, June 6, 26, 1926. Three specimens.

*Trematopygus semirufus* Cress.

At *Spartina* consocieties, 2.5 mi. south of Ames, Apr. 26, 1926, one specimen.



*Lissonota rubrica* Cress.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 31, 1925, one specimen.

*Arenetra nigrita* Cress.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 12, 1926, one specimen.

*Ephialtes tenuicornis* Cress.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 9, 1926, one specimen.

*Mesostenus americanus* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 12, 1925, one specimen.

*Vipio texanus* Cress.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, Aug. 4, 1925, two specimens.

*Trychosis rufoannulatus* Prov.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 12, 1925, one specimen.

*Hoplocryptus incertulus* Cush.

At *Spartina* consocieties, 2.5 mi. south of Ames, May 12, 1926, one specimen.

*Phaeogenes helvolus* Cress.

At *Spartina* consocieties, 2.5 mi. south of Ames, June 26, 1926, one specimen.

*Amblyteles suturalis* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 7, 1926, one specimen.

*Amblyteles volens* Cress.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 19, 1925, one specimen. At lights, *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 3, 1926, one specimen.

*Rhodites utahensis* Bass.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 21, 1926, one specimen.

*Antistrophus silphii* G. U.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10, 1925, one specimen.

*Euplectrus comstocki* How.

Reared from two *Geometridae* larvae which were brought from *Stipa spartea*—*Andropogon scoparius* association, June 4, 1926. The parasites left the host, June 7, and pupated within light, netlike webs. The adults appeared June 11, 1926. Sixteen specimens were obtained.

*Perilampus chrysopae* Cwfd.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 25, 1926, one specimen.

*Perilampus fulvicornis* Ashm.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 1925, one specimen.

*Perilampus hyalinus* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 24, 28, 1926, two specimens. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 26, 1928, one specimen.

*Pseudometagea schwarzi* Ashm.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 1925, one specimen.

*Haltichella xanticles* Walk.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Oct. 1, 1926, three specimens.

*Spilochalcis albifrons* Walsh.

At *Andropogon furcatus* consocieties, July 22, 1925, one specimen.

*Macroteleia macrogaster* Ashm.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 14, 1926, one specimen.

*Scelio opacus* Prov.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 25, 1925, one specimen.

*Hebropelte fuscipennis* Ashm.

At *Polygonum amphibium* societies, 2.5 mi. south of Ames, Aug. 2, 1926, one specimen.

*Pelecinus polyturator* Dru.

At *Stipa spartea*—*Andropogon scoparius* association, *Andropogon furcatus* consocieties, and *Andropogon furcatus*—*Sorghastrum nutans* associations. Earliest adult, Aug. 7, 1928; latest adult, Aug. 25, 1928. Six specimens.

*Ponera coarctata* subsp. *pennsylvanica* Buck.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Apr. 25, 1928, four specimens.

*Crematogaster lineolata* Say

Attending aphids on *Silphium laciniatum*, 2.5 mi. south of Ames, Aug. 25, 1925. At *Andropogon furcatus* consocieties, same locality, May 31, 1926. Seven specimens, in total.

*Crematogaster lineolata* var. *cerasi* Fitch

At *Spartina* consocieties, Gitchie-Manito State Park, July 24, 1926. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of

Hamburg State Park, July 30, 1926, two specimens. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1.5 mi. east of Verdi, Aug. 20, 1928, two specimens.

*Stenamma brevicorne* Emery subsp. or var.

At *Spartina* consociates, 2.5 mi. south of Ames, May 12, 1927, one specimen.

*Aphaenogaster fulva aquia* Buck.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 9, 1926, two specimens taken on the ground, and Apr. 18, 1926, two specimens taken by sweeping.

*Myrmica scabrinodis* Nyl. subsp. or var.

At *Spartina* consociates, and *Andropogon furcatus* consociates, chiefly. Earliest adult, May 2, 1928; latest adult, Sept. 15, 1928. Common.

*Myrmica brevinodis* Emery

On *Helianthus* sp., 2 mi. north of Ames, Aug. 4, 1926, one specimen.

*Myrmica scabrinodis* var. *sabuleti* Mein.

At *Andropogon furcatus* consociates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, June 30, 1928; latest adult, Aug. 5, 1927. Common.

*Myrmica scabrinodis schencki* var. *emeryana* Forel.

At *Spartina* consociates, 10 mi. southwest of Kelso, July 30, 1928, one specimen.

*Leptothorax curvispinosus* subsp. *ambiguus* Emery

At *Andropogon furcatus* consociates, 2.5 mi. south of Ames, Apr. 16, 24, 1927, two specimens.

*Leptothorax tricarinatus* Emery

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, one specimen.

*Dorymyrmex pyramicus* Rog.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, July 30, 1928; latest adult, Oct. 20, 1928. Common.

*Iridomyrmex pruinosus* Rog.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Tapinoma sessile* Say

At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon furcatus*—*Spartina Michauxiana* associates. Earliest adult, Apr. 16, 1927; latest adult, Sept. 15, 1928. Common at *Andropogon furcatus* consociates, and *Spartina* consociates.

*Prenolepis parvula* Mayr.

At *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, Aug. 5, 1927; latest adult, Sept. 15, 1928. Common.

*Lasius niger* var. *americanus* Emery

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Apr. 26, Aug. 14, 1926, two specimens.

*Lasius niger* var. *near americanus* Emery

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 22, 1925, one specimen.

*Lasius niger* var. *neoniger* Emery

At *Andropogon furcatus*—*Spartina Michauxiana* associates, *Andropogon furcatus*—*Sorghastrum nutans* associates, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 19, 1928; latest adult, Sept. 15, 1928. Common at *Stipa spartea*—*Andropogon scoparius* association and *Andropogon furcatus*—*Sorghastrum nutans* associates.

*Lasius niger alienus* var. *americanus* Emery

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, May 9, 1928, and 1.5 mi. northeast of Ocheyedon, July 23, 1928. Three specimens.

*Lasius brevicornis* Emery

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Mar. 25, 1928, three specimens.

*Lasius latipes* Walsh

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, April 25, 1926, common under stones.

*Formica cinerea* var. *neocinerea* Whlr.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Andropogon furcatus*—*Sorghastrum nutans* associates. Earliest adult, Apr. 25, 1928; latest adult, Sept. 7, 1928. Common at *Spartina* consocieties.

*Formica truncicola integroides* Emery

At *Stipa spartea*—*Andropogon scoparius* association, chiefly. Earliest adult, June 4, 1926; latest adult, Oct. 9, 1926. Attending aphids (*Aphis* sp.) on *Cirsium iowense*, 2 mi. north of Ames, Aug. 13, 1926, and *Aphis monardae* on *Monarda mollis*, same locality and date. Common.

*Formica pallide-fulva* subsp. *nitidivendris* Emery

At *Stipa spartea*—*Andropogon scoparius* association, chiefly, 6 mi. northwest of Ledyard, Aug. 7, 1928, and 7 mi. northwest of Thompson, Aug. 6, 1928, three specimens. At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1.5 mi. southeast of Muscatine, Sept. 1, 1928, one specimen.

*Formica pallide-fulva schaufussi* Mayr.

On *Silphium laciniatum*, 2.5 mi. south of Ames, July 27, 1926, five specimens. On *Cirsium iowense*, same locality, Aug. 9, 1926, two specimens. Common at *Andropogon furcatus* consocieties.



*Formica pallide—fulva schaufussi* var. near *incerta* Emery

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 25, 1926, two specimens.

*Formica pallide—fulva* var. *incerta* Emery

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Andropogon furcatus*—*Sorghastrum nutans* associates, chiefly. Earliest adult, May 12, 1927; latest adult, Sept. 16, 1928. Common at *Andropogon furcatus* consocieties.

*Formica pallide—fulva* Latr. var.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 2, 1926, one specimen.

*Formica neogagates* Emery var.

At *Bouteloua hirsuta*—*B. curtipendula* association, Ocheyedon Mound, July 23, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Buffalo Center, Aug. 6, 1928, one specimen, 5 mi. south of Stanhope, one specimen, 7 mi. northwest of Thompson, May 18, June 30, Sept. 15, 1928, three specimens, and 1.5 mi. northeast of Ocheyedon, July 23, 1928, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, one specimen.

*Formica neogagates lasioides* var. *vetula* Whlr.

At *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon scoparius*—*Bouteloua curtipendula* association, chiefly. Earliest adult, May 18, 1928; latest adult, Aug. 7, 1928. Common at *Stipa spartea*—*Andropogon scoparius* association.

*Formica neogagates* var. *vinculans* Whlr.

At *Stipa spartea*—*Andropogon scoparius* association, chiefly. Earliest adult, May 18, 1928; latest adult, Sept. 19, 1928. Common.

*Formica fusca* var. *argentea* Whlr.

At *Stipa spartea*—*Andropogon scoparius* association, chiefly. Earliest adult, July 23, 1928; latest adult, Sept. 19, 1928. Common.

*Formica fusca* var. *subsericea* Say

At *Spartina* consocieties, chiefly. Earliest adult, May 12, 1926; latest adult, Sept. 15, 1928. Common.

*Formica rufa obscuripe* var. *melanotica* Emery

At *Stipa spartea*—*Andropogon scoparius* and *Andropogon scoparius*—*Bouteloua curtipendula* associations, and *Andropogon furcatus* consocieties. Earliest adult, Apr. 12, 1926; latest adult, Sept. 19, 1928. Numerous at *Stipa spartea*—*Andropogon scoparius*, and *Andropogon scoparius*—*Bouteloua curtipendula* associations.

*Elis atriventris* Gahan

At flowers of *Eryngium yuccifolium*, 2 mi. north of Ames, July 30, 1926, one specimen.

*Elis quinquecincta* Fabr.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 31, 1926, one specimen. At *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927, one specimen. At *Spartina* consocieties, 10 mi. southwest of Kelso, July 30, 1928, one specimen. At *Solidago Riddellii*, blooming, 6 mi. south of Washington, Aug. 24, 1928, one specimen.

*Tiphia illinoensis* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 10, 1925, one specimen.

*Myrmosa unicolor* Say

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 19, 1927, one specimen.

*Pseudomethoca canadensis* Blake

At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedan Mound, July 23, 1928, one specimen. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. north of Reels City, Aug. 1, 1928, one specimen.

*Pseudomethoca* sp. near *geryon* Fox

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 7, 1926, one specimen.

*Pseudomethoca sanbornii* Blake

At *Bouteloua hirsuta*—*B. curtipendula* association, 2 mi. north of Ames, July 14, 1926, one specimen.

*Dasymutilla interrupta* Banks

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 31, 1926, one specimen.

*Dasymutilla macra* Fox

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Council Bluffs, July 31, 1928, one specimen.

*Dasymutilla sparsa* Fox

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. north of Reels City, Aug. 1, 1928, one specimen.

*Ceropales fulvipes* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 18, 1926, one specimen.

*Ageniella annecta* Bks.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, May 18, 1928, one specimen.

*Ageniella atrata* Prov.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 29, 1925, one specimen.

*Priocnemis nothus* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 25, 1926, one specimen.

*Aporinellus laticeps* Bks.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 19, 1926, one specimen.

*Psammochares relativus* Fox (?)

At *Spartina* consocieties, 2.5 mi. south of Ames, July 13, 1926, one specimen.

*Polistes variatus* Cress.

At *Silphium laciniatum* apparently gathering gum from a bruised spot on the plant, 2.5 mi. south of Ames, July 20, 1925, one specimen.

*Polistes pallipes* Le P.

From nest fastened to *Helianthus grosseserratus*, 10 mi. southwest of Ames, July 14, 1928, one specimen. From nest attached to *Amorpha canescens*, 1.5 mi. east of Verdi, Sept. 5, 1928, two specimens. Swept from flowers of *Cassia Chamaecrista*, Aug. 25, 1928, one specimen.

*Alyson trianguliferus* Prov.

At *Carex* societies, Lake Amana, June 23, 1928, one specimen.

*Nysson plagiatus* Cress.

At *Elymus virginicus*, 1 mi. south of Amana, Aug. 13, 1927, one specimen.

*Brachystegus* sp. near *trichrus* Mickel

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. north of Reels City, Aug. 1, 1928, one specimen.

*Psen cressoni* Pack.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 5, 1927, two specimens.

*Psen mellipes* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, Aug. 4, 1927, and Aug. 4, 1928, three specimens. At *Spartina* consocieties, 8 mi. southeast of Britt, Aug. 9, 1928, two specimens.

*Oxybelus unicus* Mickel (?)

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Notoglossa emarginata* Say

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 28, 1926, one specimen.

*Crabro armaticeps* Fox

At *Helianthus grosseserratus*, Lake Amana, June 23, 1928, one specimen.

*Trypoxylon frigidum* Sm.

At *Spartina consociata*, Lake Amana, Aug. 12, 1927, one specimen.

*Didineis texana* Cress.

At *Polygonum amphibium* socris, 10 mi. southwest of Kelso, July 30, 1928, one specimen.

*Ammobia ichneumonea* Linn.

At flowers of *Cirsium iowense*, 2 mi. north of Ames, Aug. 13, 1926, one specimen. At flowers of *Solidago* sp., same locality, Sept. 17, 1926, one specimen.

*Sphex pictipennis* Walsh

At flowers of *Eryngium yuccifolium*, 2 mi. north of Ames, Aug. 4, 1926, one specimen.

*Sphex procera* Klug

At *Stipa spartea*—*Andropogon furcatus* association, 2 mi. north of Ames, Sept. 17, 1926, one specimen.

*Sphex urnarius* Dahlb.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, Aug. 19, 1927, one specimen.

*Sphex varipes* Cress.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. north of Reels City, Aug. 1, 1928, one specimen.

*Sceliphron caementarius* Dru.

At flowers of *Cicuta maculata*, 2.5 mi. south of Ames, July 6, 1926, one specimen.

*Lyroda subita* Say

At flowers of *Cicuta maculata*, 2.5 mi. south of Ames, July 15, 1926, and Aug. 4, 1928, two specimens.

*Larropsis distinctus* Smith

At flowers of *Solidago Riddellii*, 6 mi. south of Washington, Aug. 24, 1928, two specimens.

*Astata unicolor* Say

At flowers of *Cicuta maculata*, 2.5 mi. south of Ames, Aug. 4, 1928, one specimen.

*Bembex sayi* Cress.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie-Manito State Park, July 24, 1928, one specimen.

*Bembex spinolae* Le P.

At *Andropogon furcatus*—*Spartina Michauxiana* socris, 6 mi. northwest of Ledyard, Aug. 7, 1928, two specimens.

*Cerceris kennicottii* Cress.

At *Spartina consociata*, .5 mi. south of Missouri Valley, Aug. 1, 1928, one specimen.



*Cerceris venator* Cress.

At rest among flowers of *Asclepias verticillata*, 2.5 mi. south of Ames, Aug. 9, 1927, one specimen.

*Cerceris* sp. near *jacunda* Cr.

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, July 10, 1925, one specimen.

*Cerceris compar* Cress. (?)

At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen.

*Halictus provancheri* D. T.

At *Andropogon furcatus* consocieties, *Stipa spartea*—*Andropogon scoparius* association, *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Bouteloua hirsuta*—*Bouteloua curtipendula* association. Earliest adult, May 11, 1926; latest adult, Oct. 1, 1926. One specimen at flowers of *Zizia aurea*, and one at flowers of *Fragaria virginiana*. Common at *Bouteloua hirsuta*—*B. curtipendula*, and *Stipa spartea*—*Andropogon scoparius* associations.

*Halictus ligatus* Say

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 19, Aug. 9, 1926, and July 10, 1928; two specimens at flowers of *Silphium laciniatum*, and one at flowers of *Heliopsis scabra*. At flowers of *Silphium laciniatum*, 1 mi. south of Amana, July 20, 1928, one specimen.

*Halictus lerouxi* Le P.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 25, 1925, and May 7, 1926, two specimens. At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, June 29, 1926, one specimen.

*Halictus coriaceus* Smith

At flowers of *Anemone patens* var. *Wolfgangiana*, 2 mi. north of Ames, Apr. 21, 1926. At *Stipa spartea*—*Andropogon scoparius* association, same locality, May 9, 1926, one specimen.

*Halictus* sp. near *arcuatus* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 25, 1925, Aug. 4, 1926, two specimens.

*Halictus tegularis* Robt.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, and 1 mi. north of Reels City, Aug. 1, 1928, two specimens. At *Polygonum amphibium* societies, .5 mi. south of Missouri Valley, Aug. 1, 1928, one specimen. At flowers of *Cassia Chamaecrista*, 1 mi. south of Amana, Aug. 25, 1928, one specimen.

*Halictus pilosus* Sm.

At *Andropogon furcatus* consocieties, *Spartina* consocieties, *Andropogon furcatus*—*Sorghastrum nutans* societies, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 12, 1926; latest adult, Sept. 1,

1928. Taken at flowers of *Cicuta maculata*, *Pedicularis canadensis*, *Anemone patens* var. *Wolfgangiana*, *Rosa pratincola*, and *Coreopsis palmata*. Common.

*Halictus pruinosis* Robt.

At *Andropogon furcatus* consocieties, *Spartina* consocieties, and *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 17, 1926; latest adult, Oct. 1, 1926. Taken at flowers of *Zizia aurea*, one specimen. Common at *Andropogon furcatus* and *Spartina* consocieties.

*Halictus zephyrus* Smith

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Oct. 1, 1926, one specimen.

*Halictus nymphaeae* Robt.

At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928, three specimens. At *Andropogon furcatus* consocieties, 5 mi. northwest of Buffalo Center, Sept. 16, 1928, two specimens.

*Halictus albipennis* Robt.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, *Andropogon furcatus*—*Sorghastrum nutans* associates, *Stipa spartea*—*Andropogon scoparius* association, and *Bouteloua hirsuta*—*B. curtipendula* association. Earliest adult, May 15, 1926; latest adult, Oct. 1, 1926. Several specimens taken at flowers of *Rosa pratincola*, *Silphium laciniatum*, and *Veronica virginica*. Common at *Bouteloua hirsuta*—*B. curtipendula* association, *Stipa spartea*—*Andropogon scoparius* association, and *Andropogon furcatus* consocieties.

*Halictus sparsus* Robt.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, and *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, May 17, 1926; latest adult, Aug. 1, 1928. Common at *Andropogon scoparius*—*Bouteloua curtipendula* association.

*Halictus versatus* Robt.

At flowers of *Silphium laciniatum*, and *Liatris pycnostachya*, 2.5 mi. south of Ames, July 27-Aug. 5, 1926, five specimens. At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, one specimen.

*Halictus coreopsis* Robt.

At flowers of *Heliopsis scabra*, 2.5 mi. south of Ames, July 10, 1928, one specimen.

*Halictus pictus* Cwfd.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Halictus unicus* Sandhouse

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. west of Hamburg State Park, July 30, 1928, one specimen.

*Paralictus cephalicus* Robt.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Agapostemon texanus* Cress.

At flowers of *Eryngium yuccifolium*, 2 mi. north of Ames, July 21, 1926, one specimen, and at flowers of *Silphium laciniatum*, July 29, 1926, one specimen. At flowers of *Silphium laciniatum*, 2.5 mi. south of Ames, July 29, 1926, two specimens. At flowers of *Oenothera serrulata*, 7.75 mi. northwest of Thompson, June 30, 1928, one specimen.

*Agapostemon texanus iowensis* Ckll.

At *Andropogon furcatus* consociates, 2.5 mi. south of Ames, May 21, July 27, 1926, three specimens.

*Agapostemon virescens* Fabr.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 25, Oct. 1, 1926, and at flowers of *Silphium laciniatum*, same locality, June 21, 1926; four specimens.

*Augochlora confusa* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 21, 1926, two specimens.

*Augochlora pura* Say

At *Stipa spartea*—*Andropogon scoparius* association, *Andropogon furcatus* consociates, and *Spartina* consociates. Earliest adult, May 11, 1926; latest adult, Oct. 1, 1926. Taken at flowers of *Cirsium iowense*, *Silphium laciniatum*, *Eryngium yuccifolium*, and *Rosa pratincola*. One bee had been caught by an ambush bug (*Phymata erosa fasciata* Gray) on flowers of *Eryngium yuccifolium*, 2 mi. north of Ames, July 29, 1926.

*Sphecodes arvensis* Patt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 1, 1926, one specimen.

*Sphecodes illinoiensis* Robt.

At flower of *Zizia aurea*, 2.5 mi. south of Ames, May 17, 1926, one specimen, and at *Stipa spartea*—*Andropogon furcatus* association, 2 mi. north of Ames, Oct. 1, 1926, one specimen.

*Protandrena asclepiadis* Ckll.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, three specimens, and 1 mi. west of Hamburg State Park, July 30, 1928, one specimen.

*Andrena crawfordi* Vier.

At flowers of *Rosa pratincola*, 2 mi. north of Ames, June 22, 1926, one specimen.

*Andrena cressoni* Robt.

At flowers of *Ranunculus septentrionalis*, 2.5 mi. south of Ames, May 21, 1927, two specimens. At *Spartina* consociates, same locality, May 20,

1927, one specimen, and at *Andropogon furcatus* consocieties, May 25, 1926, one specimen.

*Andrena flavoclypeata* Smith

At *Andropogon furcatus* consocieties, 2.5 mi. south of Ames, May 19, 1926, one specimen.

*Macropis steironematis* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 23, 1925, one specimen.

*Macropis patellata* Patt.

At flowers of *Cicuta maculata*, 2.5 mi. south of Ames, July 6, 1926, one specimen.

*Perdita punctata* Ckll. (?)

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 23, 1925, one specimen.

*Panurginus innuptis* Ckll.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 28, 1926, one specimen.

*Calliopsis andreniformis* Sm.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mi. north of Reels City, Aug. 1, 1926, one specimen.

*Nomada* sp. near *incerta* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 5, 1926, one specimen.

*Nomada* sp. near *illinoiensis* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 25, 1926, one specimen.

*Nomada articulata* Smith

At *Andropogon furcatus* consocieties, 2 mi. north of Ames, July 2, 1926, one specimen.

*Nomada* sp. near *superba* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, May 20, 1926, one specimen.

*Epeolus* sp. near *autumnalis* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 7, 1925, one specimen.

*Pyrrhomelecta bifasciata* Cress.

At *Stipa spartea*—*Bouteloua curtipendula* association, 2 mi. north of Ames, July 6, 18, 1925. At flowers of *Lepachys pinnata*, July 31, 1926, one specimen.



*Tripeolus remigatus* Fabr.

At *Andropogon furcatus* consociés, 2 mi. north of Ames, Aug. 5, 1925, one specimen.

*Melissodes cnici* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 6, 1928, one specimen.

*Melissodes obliqua* Say

At flower of *Silphium laciniatum*, 2.5 mi. south of Ames, July 27, 1926, one specimen. At *Andropogon furcatus* consociés, same locality, Aug. 17, 1926, one specimen. At flowers of *Rudbeckia hirta*, 1 mi. south of Amana, July 20, 1928, one specimen.

*Melissodes* sp. near *pallidicincta* Ckll.

At flowers of *Solidago* sp., 2 mi. north of Ames, Sept. 24, 1926, one specimen.

*Melissodes rustica* Say

At *Bouteloua hirsuta*—*B. curtipendula* association, 2 mi. north of Ames, July 11, 1928, one specimen.

*Melissodes pennsylvanica* Lep.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, July 7, 1927, one specimen. At flowers of *Silphium laciniatum*, gathering pollen, same locality, Aug. 9, 1927, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 27, 1926, two specimens, one of which had been caught by an ambush bug (*Phymata erosa fasciata* Gray).

*Melissodes semiagilis* Ckll.

At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. northwest of Thompson, July 7, 1928, one specimen.

*Tetralonia atriventris* Smith

At flowers of *Cirsium iowense*, 2 mi. north of Ames, Aug. 9, 25, 1926, eight specimens.

*Tetralonia frater* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 18, 22, 1926, two specimens.

*Tetralonia speciosa* Cress.

At *Andropogon furcatus* consociés, May 28, 31, June 19, 25, 1926, five specimens. At *Stipa spartea*—*Andropogon scoparius* association, 5 mi. south of Stanhope, June 15, 1928, one specimen.

*Hylaeus ziziae* Robt.

At *Andropogon furcatus* consociés, 2.5 mi. south of Ames, July 16, 1925, one specimen.

*Colletes eulophi* (?) Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 20, 1925, one specimen, and June 28, 1926, two specimens.

*Colletes latitarsis* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, July 28, 1925, one specimen.

*Colletes americanus* Cress.

At flowers of *Cicuta maculata*, 5 mi. northwest of Buffalo Center, July 7, 1928, one specimen.

*Colletes willistoni* Robt.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 15 mi. north of Sioux City, July 25, 1928, one specimen.

*Megachile brevis* Say

At flowers of *Liatris pycnostachya*, 2.5 mi. south of Ames, Aug. 11, 1927, one specimen. At *Apocynum androsaemifolium* family, 1 mi. south of Amana, June 23, 1928, one specimen.

*Megachile perbrevis* Cress.

At flowers of *Cicuta maculata*, 2.5 mi. south of Ames, July 6, 1926, one specimen. At flowers of *Liatris* sp., same locality, Aug. 2, 1926. At *Andropogon furcatus* consociates, same locality, June 26, July 26, 1926, one specimen.

*Megachile latimana* Say

At *Andropogon furcatus* consociates, on flowers of *Cirsium iowense*, 2 mi. north of Ames, Aug. 9, 1926, one specimen.

*Megachile inimica* Cress.

At *Bouteloua hirsuta*—*B. curtipendula* association, 5 mi. south of Stanhope, Sept. 19, 1928, one specimen.

*Holcopasites haematurus* Ckll. & Hicks

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, two specimens.

*Coelioxys sayi* Robt.

At *Stipa spartea*—*Andropogon scoparius* association, Sept. 24, 1926, one specimen. At *Andropogon furcatus* consociates, 2.5 mi. south of Ames, Aug. 11, 1925, one specimen.

*Ceratina dupla* Say

At *Bouteloua hirsuta*—*B. curtipendula* association, 2 mi. north of Ames, Apr. 18, 1927, one specimen.

*Bombus pennsylvanicus* De G.

At *Andropogon furcatus* consociates, 2.5 mi. south of Ames, Aug. 11, 1925, May 25, 1926, two specimens. At flowers of *Cirsium iowense*, 2 mi. north of Ames, Sept. 24, 1926. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, Aug. 13, 1926, one specimen.

*Bombus auricomus* Robt.

At flowers of *Brauneria purpurea*, 2 mi. north of Ames, June 22, 1926, one specimen. At flowers of *Cirsium iowense*, same locality, Aug. 13, 1926,

one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 2 mi. north of Ames, June 19, 22, 1926, three specimens.

*Bombus separatus* Cress.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mi. south of Amana, June 23, 1928, and Lacey-Keosauqua State Park, Aug. 22, 1928, two specimens. At flowers of *Asclepias purpurascens*, 2.5 mi. south of Ames, June 16, 1928, one specimen, and *Andropogon furcatus* consociates, same locality, May 12, 1927, two specimens. At *Spartina* consociates, Lake Amana, Aug. 12, 1927, two specimens.

*Bremus fervidus* Fabr.

At *Andropogon furcatus* consociates, 2 mi. south of Ledyard, May 9, 1926, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 7.75 mi. northwest of Thompson, May 18, 1928, one specimen.

*Bremus americanorum* Fabr.

At flowers of *Cassia Chamaecrista*, Aug. 12, 1927, 1 mi. south of Amana, four specimens.

*Apis mellifera* Linn.

At *Stipa spartea*—*Andropogon scoparius* association, northeast of Iowa State College grounds, July 11, 1928, one specimen. Other specimens have been observed at several communities.

# SUMMARY

1. During the years 1925 to 1928, inclusive, insects representing 1175 determined species, subspecies and varieties were collected at 8 typical plant communities of less disturbed prairie, occurring at 40 localities in several parts of Iowa. Approximately 15,000 specimens were collected during the course of this work.

2. The following list contains the more common and numerous species at each plant community. The figure after a species signifies the number of other communities at which it was found to be common or numerous.

*Bouteloua hirsuta*—*B. curtipendula* (mesquite grass) association

## Order Orthoptera

*Eritettix simplex*, *Ageneotettix deorum* (1), *Dissosteira carolina* (1), *Mestobregma kiowa kiowa* (1), *Encoptolophus sordidus*, *Hippiscus apiculatus*, *Melanoplus dawsoni* (2), *Phoetaliotes nebrascensis*.

## Order Hemiptera

*Nysius ericae* (1), *Geocoris uliginosus limbatus* (3), *Ligyrocoris diffusus* (6), *Nabis fesus* (4), *Adelphocoris rapidus* (5), *Lygus pratensis* (2).

## Order Homoptera

*Deltocephalus stylatus*, *Phlepsius altus* (1), *Scolops angustatus* (1).

## Order Coleoptera

*Cicindela punctulata*, *Hippodamia tredecim-punctata* (7), *H. par-enthesis* (7), *Aphodius distinctus* (6), *Trirhabda virgata* (4), *Dia-brotica duodecimpunctata* (5), *D. longicornis* (4).

## Order Diptera

*Mesogramma marginata* (7).

## Order Hymenoptera

*Dorymyrmex pyramicus* (1), *Halictus provancheri* (2), *H. albi-pennis* (2).

*Andropogon scoparius*—*Bouteloua curtipendula* (beard grass—mesquite grass) association.

## Order Orthoptera

*Diapheromera veliei* (3), *Pseudopomala brachyptera*, *Mermiria maculipennis macclungi*, *Ageneotettix deorum* (1), *Dissosteira carolina* (1), *Mestobregma kiowa kiowa* (1), *Hesperotettix pratensis*, *Melanoplus dawsoni* (2), *M. mexicanis atlanis*, *M. femur-rubrum* (4), *M. keeleri luridus* (3), *Arethaea gracilipes constricta*, *Conocephalus saltans* (3), *Nemobius fasciatus* (2), *Gryllus assimilis* (2), *Oceanthus nigricornis argentinus*.

## Order Hemiptera

*Corimelaena agrella*, *Coenus delius* (1), *Neottiglossa sulcifrons*, *Ortholomus scolopax*, *Geocoris uliginosus limbatus* (3), *Ligyro-coris diffusus* (5), *Phymata erosa fasciata* (4), *Sinea diadema* (1), *Nabis ferus* (4), *Triphleps insidiosus* (3), *Adelphocoris rap-idus* (5), *Polymerus basalis*, *Lygus pratensis* (2), *Coquillettia mimetica*, *Ilacora divisa* (2).

## Order Homoptera

*Philaronia bilineata*, *Stictocephala inermis* (1), *Vanduzeei trigul-tata* (1), *Agallia sanguinolenta* (3), *Oncometopia lateralis*, *Gypona octolineata*, *Xerophloea viridis*, *Platymetopius cinereus*, *Driotura robusta*, *Euscelis anthracinus*, *E. striatulus* (1), *Phlep-sius altus* (1), *Chlorotettix spatulatus*, *Scolops sulcipes* (3), *S. osborni*, *S. angustatus* (1), *S. pungens*, *Bruchomorpha dorsata*, *Acanalonia bivittata*, *Ormenis pruinosa*, *Pissonotus delicatus*.

## Order Coleoptera

*Collops tricolor* (1), *Hippodamia tredecim-punctata* (7), *H. par-enthesis* (7), *H. convergens* (6), *Coccinella novemnotata* (6), *Tetraopes femoratus amnicola*, *Nodonota tristis*, *Graphops varians*, *Trirhabda virgata* (4), *Anthonomus squamosus*.

## Order Lepidoptera

*Cercyonis alope olympus* (3).

## Order Diptera

*Psilocephala frontalis* (1), *Dolichopus bifractus* (6), *Mesogramma marginata* (7), *Coenosia lata* (6), *Ensina humilis* (4), *Euaresia bella* (6).



Order *Hymenoptera*

*Dorymyrmex pyramicus* (1), *Formica rufa obscuripe* var. *melanotica* (1), *Halictus provancheri* (2), *H. sparsus*.

*Stipa spartea*—*Andropogon scoparius* (porcupine grass—beard grass) association.

Order *Orthoptera*

*Diapheromera veliei* (3), *Orphulella speciosa*, *Chortophaga viridifasciata*, *Melanoplus dawsoni* (2), *M. femur-rubrum* (4), *M. keeleri luridus* (3), *Conocephalus saltans* (2), *Nemobius fasciatus* (2), *Gryllus assimilis* (2), *Oecanthus nigricornis quadripunctatus*.

Order *Hemiptera*

*Homaemus bijugis*, *Peribalus limbolarius* (2), *Trichopepla atricornis* (1), *Euschistus variolarius* (2), *Coenus delius* (1), *Alydus conspersus*, *A. pilosulus*, *Nysius ericae* (1), *Geocoris uliginosus limbatus* (3), *Sphaerobius insignis*, *Ligyrocoris diffusus* (5), *Zeridoneus costalis*, *Pseudocnemodus canadensis*, *Atheus mimeticus*, *Phymata erosa fasciata* (4), *Sinea diadema* (1), *Nabis subcoleoptratus*, *N. ferus* (4), *Triphleps insidiosus* (3), *Adelphocoris rapidus* (5), *Lygus pratensis* (2), *Lygus pratensis oblineatus* (3), *Lopidea media* (2), *L. teton*, *Ilnacora divisa* (2).

Order *Homoptera*

*Melampsalta calliope*, *Stictocephala inermis* (1), *Vanduzeeia tri-guttata* (1), *Publilia modesta*, *Campylenchia latipes*, *Agallia sanguinolenta* (2), *Draeculacephala mollipes* (2), *Parabolocratus viridis*, *Deltocephalus configuratus*, *D. inimicus* (2), *D. unicoloratus*, *Athysanella magdalena*, *Euscelis striatulus* (1), *E. comma* (2), *Chlorotettix unicolor*, *Scolops sulcipes* (3), *Aphalara veaziei*.

Order *Coleoptera*

*Eumolops sodalis*, *Chauliognathus pennsylvanicus* (2), *Epicaula trichrus*, *E. pennsylvanica* (2), *Phalacrus politus* (2), *Hippodamia tredecim-punctata* (7), *H. parenthesis* (7), *H. convergens* (6), *Coccinella novemnotata* (6), *Aphodius distinctus* (6), *Antipus laticlavus*, *Pachybrachys luridus*, *Nodonota puncticollis*, *Colaspis brunnea*, *Graphops curtipennis* (1), *Trirhabda virgata* (4), *Galerucella cribrata*, *Diabrotica duodecimpunctata* (4), *D. longicornis* (4), *Oedionychis thyamoides* (2), *Bruchus fraterculus*, *Rhynchites bicolor*, *Apion varicorne*, *Anametis granulata*, *Epicaerus imbricatus*, *Sitona tibialis*, *Baris striata*.

Order *Lepidoptera*

*Cercyonis alope olympus* (3), *Nephelodes albilinea* (1), *Papaipema arctivorens*, *P. cataphracta*, *P. eryngii*, *Crambus* sp. (1), *Tetralopha dolorosella* (2), *Sparganothis pallorana* (1).

Order *Diptera*

*Aedes vexans* (4), *Psilocephala frontalis* (1), *Leptogaster murinus*, *Promachus vertebratus* (2), *Dolichopus bifractus* (6), *Mesogramma marginata* (7), *Sphaerophoria cylindrica* (3), *Hylemyia cili-*

*crura*, *Coenosia lata* (6), *Limnia saratogensis* (5), *Ensina humilis* (4), *Euaresta bella* (6), *Meromyza americana* (1), *Diplotoxa versicolor*, *Ectecephala similis*, *Pholeomyia indecora*.

#### Order Hymenoptera

*Euplectrus comstocki*, *Myrmica scabrinodis* var. *subleti* (1), *Prenolepsis parvula*, *Lasius niger* var. *neoniger* (1), *L. latipes*, *Formica truncicola integroides*, *F. neogagates lasiodes* var. *vetula*, *F. neogagates* var. *vinculans*, *F. fusca* var. *argentea*, *F. rufa obscuripes* var. *melanotica* (1), *Halictus provancheri* (2), *H. pilosus* (3), *H. albipennis* (2), *Tetralonia atriventris*, *Bombus auricomus*.

*Andropogon furcatus*—*Sorghastrum nutans* (beard grass—wood grass) associates.

#### Order Orthoptera

*Diapheromera veliei* (3), *Melanoplus femur-rubrum* (4), *M. keeleri luridus* (3), *Conocephalus strictus* (1), *C. saltans* (3), *Oecanthus nigricornis quadripunctatus* (2).

#### Order Hemiptera

*Galgupha atra* (1), *Peribalus limbolarius* (2), *Euschistus variolarius* (2), *Alydus eurinus*, *Phlegyas abbreviatus*, *Ligyrocoris diffusus* (5), *Phymata erosa fasciata* (4), *Nabis fesus* (5), *Triphleps insidiosus* (3), *Adelphocoris rapidus* (5), *Lygus pratensis oblineatus* (3), *Lopidea media* (2), *Plagiognathus politus* (1).

#### Order Homoptera

*Publilia concava*, *Agallia sanguinolenta* (4), *Draeculacephala mollipes* (3), *Gypona melanota* (2), *Platymetopius frontalis*, *Deltocephalus sayi*, *D. inimicus* (3), *Euscelis comma* (3), *Scolops sulcipes* (4), *Aphelonema simplex* (1), *Acanalonia bivittata* (1).

#### Order Coleoptera

*Triaena angustata*, *Chaubiognathus pennsylvanicus* (2), *Epicauta pennsylvanica* (2), *Phalacrus politus*, *Hippodamia tredecimpunctata* (7), *H. parenthesis* (7), *H. convergens* (6), *Coccinella novemnotata* (6), *C. sanguinea* (1), *Aphodius distinctus* (6), *Lema collaris*, *Nodonota convexa*, *Paria canella aterrima* (1), *P. canella sellata*, *Trirhabda virgata* (4), *Diabrotica duodecimpunctata* (4), *D. longicornis* (4), *Oedionychis thymoides* (2), *Brachytarsus sticticus*, *Odontocorynus salebrosus* (1).

#### Order Lepidoptera

*Cercyonis alope olympus* (3), *Papaipema sciata* (1), *Eupithecia miserulata*, *Tetralopha dolorosella* (2), *Sparganothis pallorana* (1).

#### Order Diptera

*Promachus vertebratus* (2), *Dolichopus bifractus* (6), *Mesogramma marginata* (7), *Coenosia lata* (6), *Limnia saratogensis* (5), *Euaresta bella* (6).

#### Order Hymenoptera

*Lasius niger* var. *neoniger* (1), *Halictus pilosus* (3).

*Andropogon furcatus* (beard grass) consociates.

## Order Orthoptera

*Diapheromera veliei* (3), *Acrydium ornatum* (1), *Tettigidea lateralis parvipennis* (1), *Orphulella speciosa* (1), *Chorthippus curtipennis* (1), *Chorthippa viridifasciata* (1), *Melanoplus femur-rubrum* (4), *M. keeleri luridus* (3), *M. differentialis* (1), *Scudderia texensis* (1), *Amblycorypha rotundifolia brachyptera*, *Neoconocephalus ensiger*, *Conocephalus fasciatus* (1), *C. strictus* (1), *C. saltans* (3), *Nemobius fasciatus* (2), *Gryllus assimilis* (2), *Oecanthus nigricornis quadripunctatus* (2).

## Order Hemiptera

*Corimelaena pulicaria* (2), *Peribalus limbolarius* (2), *Trichopepla atricornis* (1), *Mormidea lugens* (1), *Euschistus variolarius* (2), *Nysius californicus*, *Geocoris uliginosus limbatus* (3), *Ligyrocoris diffusus* (6), *Phymata erosa fasciata* (4), *Nabis fesus* (4), *Triphleps insidiosus* (3), *Adelphocoris rapidus* (5), *Lygus pratensis oblineatus* (3), *Lopidea media* (2), *Ilacora stalii* (1), *Plagiognathus politus* (1).

## Order Homoptera

*Okanagana balli*, *Ceresa diceros*, *Acutalis semicrema*, *Agallia sanguinolenta* (3), *Draeculacephala mollipes* (2), *Gypona melanota* (1), *Dorycephalus platyrhynchus*, *Mesamia nigradorsum*, *Deltoccephalus inimicus* (2), *Euscelis exitiosus*, *E. comma* (2), *Cicadula sexnotata*, *Scolops sulcipes* (3), *Aphelonema histrionica*, *Bipersona torticauda*.

## Order Coleoptera

*Dyschirius globulosus*, *Lebia pumila* (1), *Stenolophus conjunctus* (1), *Lucidota nigricans* (1), *Chauliognathus pennsylvanicus* (2), *Collops quadrimaculatus*, *Epicauta pennsylvanica* (2), *Limonium propeus*, *Melanotus cribulosus*, *Acmaeodera pulchella*, *Carpophilus brachypterus*, *Languria mozardi*, *Phalacrus politus* (2), *Hyperaspis undulata*, *Hippodamia tredecim-punctata* (7), *H. parenthesis* (7), *H. convergens* (6), *Coccinella novemnotata* (6), *C. sanguinea* (1), *Aphodius distinctus* (6), *Typocerus sinuatus*, *Graphops pubescens*, *G. curtipennis* (1), *Trirhabda virgata* (4), *Diabrotica duodecimpunctata* (4), *D. longicornis* (4), *Oedionychis thymoides* (2), *Systema elongata*, *Longilarsus testaceus* (1), *Psylliodes punctulata*, *Microrhopala vittata*, *Baris deformis*, *Odontocorynus Sulcibrosus* (1).

## Order Lepidoptera

*Cercyonis alope olympus* (3), *Nephelodes albilinea* (1), *Papaipema sciata* (1), *P. necopina*, *Tarachidia candefacta*, *Crambus elegans*, *Crambus* sp. (1), *Tetralopha dolorosella* (2), *Olethreutes hebesana*, *Sparganothis sulfureana*.

## Order Diptera

*Limonia longipennis*, *Tanytarsus nigripalpus* (1), *Aedes vexans* (4), *Exoprosopa fasciata*, *Holcocephala abdominalis*, *Promachus*

*vertebratus* (2), *Asilus erythrocnemius*, *A. paropus*, *Dolichopus bifractus* (6), *Empis nuda* (1), *Platychirus immarginatus* (1), *Allograpta obliqua*, *Mesogramma marginata* (7), *Sphaerophoria cylindrica* (3), *Coenosia lata* (6), *Schoenomyza dorsalis* (3), *Limnia saratogensis* (5), *Ensina humilis* (4), *Euaresta bella* (6), *Scatella stagnalis* (1), *Meromyza americana* (1).

#### Order Hymenoptera

*Pachynematus auratus*, *Crematogaster lineolata*, *Myrmica scabrinodis* subsp. or var. (1), *M. scabrinodis* var. *sabuleti* (1), *Tapinoma sessile* (1), *Formica pallide—fulva schaufussi*, *F. pallide—fulva* var. *incerta*, *Halictus pilosus* (3), *H. albipennis* (2), *H. pruinosis* (1), *H. versatus*.

*Spartina* (slough grass) consocieties.

#### Order Orthoptera

*Acrydium ornatum* (1), *Tettigidea lateralis parvipennis* (1), *Chorthippus curtipennis* (1), *Melanoplus femur-rubrum* (4), *M. differentialis* (1), *Scudderia texensis* (1), *Orchelimum vulgare*, *O. nigripes*, *O. concinnum*, *Conocephalus fasciatus* (1), *C. attenuatus*, *Anaxipha exigua*.

#### Order Hemiptera

*Galgupha atra* (1), *Corimelaena pulicaria* (2), *Mormidea lugens* (1), *Podisus maculiventris* (1), *Protenor belfragei*, *Corizus bohemani* (1), *Lygaeus bicrucis*, *Ischnodemus falicus*, *Ligyrocoris diffusus* (5), *Oedancala dorsalis*, *Phymata erosa fasciata* (4), *Nabis fesus pallidipennis*, *Trigonotylus tarsalis*, *Teratocoris discolor*, *Adelphocoris rapidus* (5), *Lygus pratensis oblineatus* (3), *L. campestris*, *Ilacora divisa* (2), *I. stali* (1).

#### Order Homoptera

*Cicadella hieroglyphica* var. *dolobrata*, *Draeculacephala noveboracensis*, *Kelisia crocea*, *Liburnia* near *osborni*.

#### Order Coleoptera

*Lebia viridis*, *Lebia pumila* (1), *Calleida punctata*, *Harpalus pleuriticus*, *Pseudamphasia sericea*, *Stenolophus conjunctus* (1), *Lucidota nigricans* (1), *Cantharis tantillus*, *Phalacrus politus* (2), *Coccidula lepida*, *Megilla maculata*, *Hippodamia tredecimpunctata* (7), *H. parenthesis* (7), *H. convergens* (6), *Coccinella novemnotata* (6), *Aphodius distinctus* (6), *Colaspis favosa* (1), *Paria canella aterrima* (1), *P. canella quadripunctata*, *Chrysochus auratus*, *Diabrotica duodecimpunctata* (4), *D. longicornis* (4), *Chalcoides fulvicornis nana*, *Longitarsus testaceus* (1), *Psylliodes punctulata*, *Hypera punctata*.

#### Order Lepidoptera

*Estigmene acraea* (1), *Ceramica picta* (1), *Cirphis unipuncta*, *Macronoctua onusta*, *Luperina stipata*, *Papaipema marginidens*, *Pyrausta futilalis*.



Order *Diptera*

*Helobia hybrida*, *Chironomus crassicaudata* (1), *C. lobiferus*, *Camptocladius byssinus* (1), *Cricotopus trifasciatus*, *C. trifasciatus* var. *tricinctus*, *Tanytarsus nigripalpus* (1), *Aedes vexans* (4), *Tabanus costalis*, *Psilopodinus siphon*, *Sciapus flavipes*, *Dolichopus bifractus* (6), *D. comatus*, *D. ramifer*, *Pelastoneurus vagans*, *Empis nuda* (1), *Platychirus immarginatus* (1), *Mesogramma marginata* (7), *Sphaerophoria cylindrica* (3), *Exorista nigripalpis*, *Coenosia lata* (6), *Schoenomyza dorsalis* (3), *Leptocera fontinalis*, *Tetanocera elata*, *Dictya umbrarum*, *Limnia saratogensis* (5), *Rivellia viridulans*, *Melieria ochricornis*, *Chaetopsis aenea*, *Ensina humilis* (4), *Euaresta bella* (6), *Sepsis violacea*, *Scatella stagnalis* (1).

Order *Hymenoptera*

*Myrmica scabrinodis* subsp. or var. (1), *Tapinoma sessile* (1), *Formica cinerea* var. *neocinerea*, *F. fusca* var. *subsericea*, *Halictus pilosus* (3), *H. pruinus* (1).

*Carex* (sedge) species.

Order *Homoptera*

*Thamnotettix melanogaster*, *Aphalara calthae*.

Order *Coleoptera*

*Hippodamia tredecim-punctata* (7), *H. parenthesis* (7), *H. convergens* (6), *Coccinella novemnotata* (6), *Aphodius distinctus* (6).

Order *Diptera*

*Chironomus crassicaudata* (1), *Camptocladius byssinus* (1), *Aedes vexans* (4), *Dolichopus bifractus* (6), *Mesogramma marginata* (7), *Coenosia lata* (6), *Schoenomyza dorsalis* (3), *Limnia saratogensis* (5), *Ensina humilis* (4), *Euaresta bella* (6).

*Polygonum amphibium* (smartweed) species.

Order *Hemiptera*

*Podisus maculiventris* (1), *Corimelaena pulicaria* (2), *Corizus bohemani* (1), *Adelphocoris rapidus* (6), *Deraeocoris histrio*.

Order *Homoptera*

*Ceresa bubalus*, *Phlepsius irroratus*.

Order *Coleoptera*

*Hippodamia tredecim-punctata* (7), *H. parenthesis* (7), *H. convergens* (6), *Coccinella novemnotata* (6), *Aphodius distinctus* (6), *Colaspis favosa* (1), *Diabrotica atripennis fossata*, *Systema frontalis*, *Lixus mucidus*, *L. terminalis*, *Ceutorhynchus sulcipennis*, *Rhinoncus pericarpus*.

Order *Lepidoptera*

*Estigmene acraea* (1), *Ceramica picta* (1).

Order *Diptera*

*Aedes vexans* (4), *Dolichopus bifractus* (6), *Mesogramma marginata* (7), *Sphaerophoria cylindrica* (2), *Coenosia lata* (6), *Schoenomyza dorsalis* (3), *Limnia saratogensis* (5), *Ensina humilis* (4), *Euaresta bella* (6).

3. Each of several plant communities was inhabited by a few species of insects which occurred in larger numbers and which were seldom or never found at any other community. These insects, in the following list, are suggested as the more distinctive species at the individual communities.

*Andropogon scoparius*—*Bouteloua curtipendula* (beard grass—mesquite grass) association.

Order *Orthoptera*

*Arethaea gracilipes constricta* (*Tettigoniidae*).

Order *Homoptera*

*Oncometopia lateralis* (*Cicadellidae*), *Pissonotus delicatus* (*Fulgoridae*).

*Stipa spartea*—*Andropogon scoparius* (poreupine grass—beard grass) association.

Order *Hemiptera*

*Alydus conspersus* (*Coreidae*), *Atheas mimeticus* (*Tingitidae*), *Lopidea teton* (*Miridae*).

Order *Homoptera*

*Deltocephalus configuratus* (*Cicadellidae*).

Order *Coleoptera*

*Anametis granulata* (*Curculionidae*), *Epicaerus imbricatus* (*Curculionidae*), *Sitona tibialis* (*Curculionidae*).

Order *Lepidoptera*

*Papaipema eryngii* (*Noctuidae*).

*Andropogon furcatus* (beard grass) consocieties.

Order *Orthoptera*

*Amblycorypha rotundifolia brachyptera* (*Tettigoniidae*).

Order *Coleoptera*

*Systema elongata* (*Chrysomelidae*), *Microrhopala vittata* (*Chrysomelidae*), *Baris deformis* (*Curculionidae*).

Order *Hymenoptera*

*Formica pallide*—*fulva* var. *schaufussi* (*Formicidae*).

*Spartina* consocieties.

Order *Hemiptera*

*Trigonotylus tarsalis* (*Miridae*), *Ischnodemus falicus* (*Lygaeidae*).

## Order Homoptera

*Draeculacephala noveboracensis* (Cicadellidae).*Polygonum amphibium* (smartweed) socies.

## Order Hemiptera

*Deraeocoris histrio* (Miridae).

## Order Coleoptera

*Diabrotica atripennis fossata* (Chrysomelidae), *Rhinoncus pericarpus* (Curculionidae).

## LITERATURE CITED

- ADAMS, CHARLES C.  
1915. An ecological study of prairie and forest invertebrates. Ill. State Lab. Nat. Hist. Bull., 11:34-280.
- ALDRICH, J. M.  
1905. A catalogue of North American *Diptera*. Smithsonian Inst. Misc. Coll., 46, No. 1444, 680 pp.
- ALLEN, J. A.  
1871. The fauna of the prairies. The American Naturalist, 5:4-9.
- BANKS, NATHAN  
1907. Neuropteroid insects of the United States. American Entomol. Soc., Philadelphia, Pa., 53 pp.
- BARNES, WM. AND J. McDUNNOUGH  
1917. Check list of the *Lepidoptera* of Boreal America. The Authors, Decatur, Ill., 392 pp.
- BLATCHLEY, W. S.  
1920. *Orthoptera* of Northeastern America. Nature Pub. Co., Indianapolis, Ind. 784 pp.
- BRITTON, W. E.  
1916. The *Hymenoptera*, or wasp-like insects, of Connecticut. Connecticut Geol. and Nat. Hist. Survey Bull. 22, 824 pp.
- BRUMFIELD, DANIEL M.  
1919. The animal ecology of Johnson County. Univ. of Iowa Lab. Nat. Hist. Bull. 8, No. 1, 37 pp.
- CLEMENTS, FREDERIC E.  
1920. Plant indicators. Carnegie Inst. of Washington. Pub. No. 290, 388 pp.
- COMSTOCK, JOHN H.  
1924. An introduction to entomology. Comstock Pub. Co., Ithaca, N. Y. 1044 pp.
- DRAKE, CARL J.  
1928. A synopsis of the American species of *Acalypta* (Hemip.—Tingitidae). Bull. of Brooklyn Entomol. Soc., 23:1-9.
- GRAY, ASA  
1908. New manual of botany, 7th edition. American Book Co., New York, N. Y. 926 pp.
- GUTHRIE, JOSEPH E.  
1903. The *Collembola* of Minnesota. Minnesota Geol. and Nat. Hist. Survey, Zool. Series 4, 103 pp.

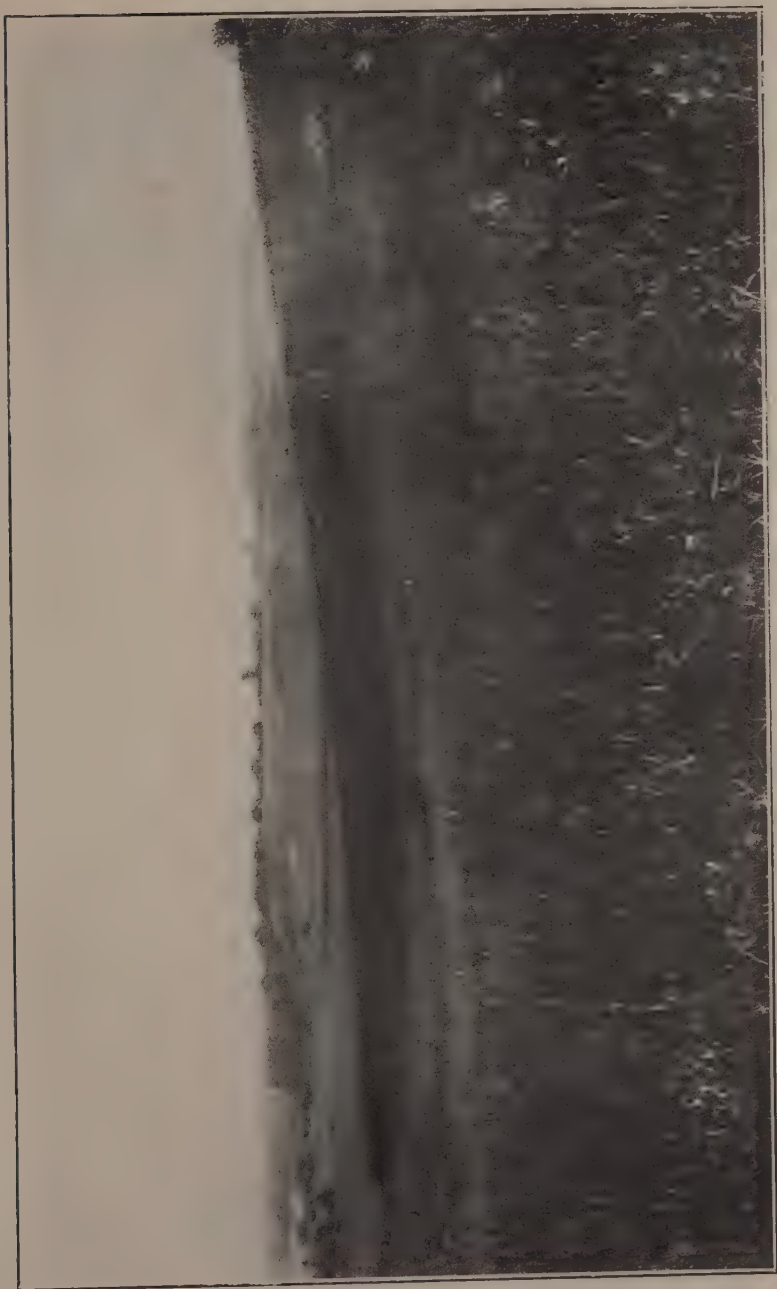
- HAYDEN, ADA  
1919. Notes of the floristic features of a prairie province. Proc. Iowa Acad. Sci., 30:369-389, 1918 (1919).
- HAYES, WILLIAM P.  
1927. Prairie insects. Ecology, 8:238-250.
- HENDRICKSON, G. O.  
1928. Some notes on the insect fauna of an Iowa prairie. Annals of Entomol. Soc. of America, 21:132-138.
- LENG, CHARLES W.  
1920. Catalogue of the *Coleoptera* of America, north of Mexico. John D. Sherman, Jr., Mount Vernon, N. Y. 470 pp.
- NEEDHAM, J. G. AND HORTENSE B. HEYWOOD  
1929. A handbook of the dragonflies of North America. Charles C. Thomas, Springfield, Ill. 378 pp.
- PAMMEL, L. H., C. R. BALL, AND F. LAMSON SCRIBNER  
1903. The grasses of Iowa, Part II. Iowa Geol. Survey, Sup. Rep. 423 pp.
- SCUDDER, SAMUEL H.  
1900. Catalogue of described *Orthoptera* of United States and Canada. Proc. of Davenport Acad. of Nat. Sci., 8, 101 pp.
- SHACKLEFORD, M. W.  
1929. Animal communities of an Illinois prairie. Ecology, 10:126-154.
- SHELFORD, VICTOR E.  
1913. Animal communities in temperate America. Geog. Soc. of Chicago Bull., 5, 362 pp.
- SHIMEK, BOHEMEL  
1911. The prairies. Univ. of Iowa Lab. Nat. Hist. Bull., 6, No. 2, 169-240.
- VAN DUZEE, EDWARD P.  
1917. Catalogue of the *Hemiptera* of America north of Mexico. Univ. of Calif. Pub., Tech. Bull., College of Agric., Agric. Expt. Sta., Entomology. 2, 902 pp.
- VESTAL, ARTHUR G.  
1913. An associational study of Illinois sand prairies. Illinois State Lab. Nat. Hist. Bull. 10, Art. I, 96 pp.
- WHELAN, DON B.  
1927. The winter fauna of the Bunch Grasses of Eastern Kansas. Ecology, 8:94-97.



## PLATE I.

In the foreground, summer aspect of *Stipa spartea*—*Andropogon scoparius* association on the Hayden farm, two miles north of Ames.

PLATE I.



## PLATE II.

*Andropogon furcatus* *Spartina Michauxiana* associates on the Templeton farm, two and one-half miles south of Ames.

PLATE II.







## CLARIFICATION OF MILK FOR AMERICAN CHEDDAR CHEESE\*

G. WILSTER\*\*

*From the Department of Dairy Industry, Iowa State College.*

Accepted for publication October 14, 1929.

The present yearly production of cheddar cheese in the world is approximately 660,000,000 pounds. On the basis of ten and one-half pounds of milk for a pound of cheese, the amount of milk used is 6,633,000,000 pounds. The yearly production of cheddar cheese in the United States is about 300,000,000 pounds, which is 45 per cent of the world's production, and 3,150,000,000 pounds of milk are required for this amount. Assuming the average wholesale price of cheese to be 22 cents a pound, the yearly value of the cheddar cheese made in this country is \$66,000,000.

The activity of certain microorganisms is essential for the ripening of cheddar cheese, and accordingly conditions of manufacturing and curing favorable for their introduction and growth must be provided. These organisms should be present in the milk at the beginning of the manufacturing process, since in their absence at this stage certain undesirable types of bacteria, which may already be present, will probably develop and cause objectionable conditions. Abnormal odors and flavors are among the common defects in cheese due to microorganisms, while a pasty body and a mealy texture also may be caused by them.

Starters containing bacteria desirable for the ripening of cheese of the cheddar type have been used for many years in an attempt to control the fermentations occurring. Oftentimes, however, a starter may not have the desirable effect because of the activity of undesirable organisms that are already present in the milk, and which find the conditions provided favorable for their growth.

Pasteurization of milk is being used successfully in some foreign countries for the control of the fermentations brought about by bacteria in cheese. At present, pasteurization is being introduced in the United States, but raw milk is used for most of the cheese that is made.

The blending and processing of cheese has been used with success in this country during recent years for the production of a more nearly uniform and more easily marketable cheese.

### HISTORICAL

Attempts to remove the foreign material which gains entrance to the milk during its production and handling are made by means of various mechanical devices. The producer usually employs the more simple of

\*A part of a thesis presented to the Graduate Faculty, Iowa State College, Ames, Iowa, June, 1928, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

\*\*It is a pleasure to acknowledge the great help given by Dr. B. W. Hammer during the period when the work here reported was in progress, as well as for his assistance in preparing the manuscript. Acknowledgment is also made to Professors M. Mortensen, E. F. Goss, N. S. Golding, and to Messrs. F. C. Hinze, H. R. Lochry and R. B. Locke for their valuable assistance.

these, such as wire screens or cotton cloths, while in the milk plants the more efficient forms such as filters and centrifugal clarifiers are used. Cream separators with the outlets so arranged that the cream and skim milk were mixed were first used for cleaning milk, while later specially constructed machines, such as clarifiers, were developed. The slime which is removed from milk by means of centrifugal force has been investigated with the object of determining the effect of the centrifugal force in a separator or clarifier on the numbers and types of bacteria present in the milk.

Doane (8), in discussing the economical methods for improving the keeping qualities of milk, states that it is doubtful who first used the separator for purifying milk, but that it is natural it should be used for this purpose, since anyone who has washed a separator has noticed a layer of matter that gathers on the inside of the steel bowl. He concluded, from some of his investigations, "that there is no doubt but that the separator removes the greater part of the dirt from milk. On the other hand, clarifying is valueless as a precaution against disease, and evidently has a tendency to cause the milk to sour more readily than when not so treated." He points out that milk which has been purified by the centrifugal methods was frequently called "clarified."

Grotenfelt (16), in studying the effect of centrifugal force on the numbers of bacteria in milk, found that in seven lots of milk centrifuging always resulted in decreases in the bacterial count. The counts on the unseparated milk ranged from 1,030 to 18,180, those on the skim milk from 220 to 11,025, and of the cream from 200 to 13,200 per c.c., while those of the separator slime ranged from 150,000 to 4,241,000 per c.c.

Hinkelmann (21) reported that from milk which had been inoculated with bouillon cultures of various organisms he was able to remove with a DeLaval clarifier from 57 to 96 per cent, with an average of 78 per cent. He believed that small or gram-positive organisms were of a greater density than the ordinary milk bacteria.

Dunbar and Kister (10), in working with a Heine cleaning centrifuge of the hollow bowl type in which there was a filter cloth, found that the running of milk through the machine caused no particular changes in the milk, as shown by the specific gravity, and solids not-fat content of the milk before and after it had been cleaned. They found many bacteria in the slime, but their conclusions indicate that the chief function of the machine lies in the removal of dirt particles and foreign matter.

Fleischmann (15) quotes Hueppe, who asserts that "most of the organisms, and among these the most dangerous ones, remain behind in the separator residue," but Fleischmann doubts that centrifuging has this effect.

Eckles and Barnes (11) were among the first to study the effect of centrifugalization of milk upon the number of bacteria present. In each of seven experiments they found that when running milk through a separator, the milk contained from 15 to 51 per cent fewer organisms after centrifuging than before. They also found that when separating milk an average of "29 per cent of the total number of organisms went into the skim milk, 24 per cent into the cream, and about 47 per cent into the slime."

Bahlman (3), in eight tests found that the unclarified milk averaged 1,312,000, and the clarified milk 1,670,000 bacteria per c.c. The increases in the counts due to clarification ranged from 9 to 60 per cent and aver-

aged 27 per cent. He states that the increases were due to the breaking apart of bacterial clumps by the mechanical action of the clarifier.

McClintock (30) studied the effect of clarification on the number of bacteria in milk in 59 trials in city plants using three different types of clarifiers. The unclarified milk contained from 54,000 to 2,650,000 bacteria per c.c. and the clarified milk from 36,000 to 12,000,000. Sometimes clarification caused an increase and sometimes a decrease in the count, but increases occurred in only three trials. McClintock (31) also clarified sterile milk which had been inoculated with lactic acid bacteria or with pathogenic organisms. Clarification resulted in a decrease of from 15.3 to 16.1 per cent in the number of acid formers and of from 95.8 to 99 per cent in the pathogenic organisms.

Hammer (17), in working with a DeLaval clarifier, found that the plate counts of the clarified milk were commonly, although not constantly, higher than those of the unclarified milk. Since the clarifier slime which he examined contained large numbers of bacteria, and contamination of the milk was excluded, he accounts for the increase in the counts as a result of clarification by the breaking up of clumps of organisms due to centrifuging. This investigator is of the opinion that "whether there will be an increase or a decrease in the apparent number of organisms during clarification probably depends on the types of organisms and on the presence of clumps." Working with a Sharples clarifier, Hammer (18) later obtained results which were in the main comparable to the earlier data.

McInerney (32) studied the effect of clarification on high and low count milk. In 28 lots of low count milk, the unclarified milk contained an average of 8,410, and the clarified milk 15,739 bacteria per c.c., the increases in the counts due to clarification varied from 1.76 to 415.1 per cent and averaged 87.15 per cent. In 17 lots of high count milk the unclarified milk contained an average of 9,778,191, and the clarified milk 21,000,694 bacteria per c.c., while the increases due to clarification ranged from 5.82 to 1574.7 and averaged 114.77 per cent.

When studying the production of sanitary milk, Sherman (38), in 15 tests found that unclarified milk contained an average of 3,640 and clarified milk an average of 7,020 bacteria per c.c.; in four tests the methylene blue test showed a quicker reduction with the clarified milk. In continuing his work he (39) later found that in 24 tests the average bacterial count of unclarified milk was 4,720 and of clarified milk 7,120 per c.c. In eight tests the unclarified milk reduced methylene blue, on an average, in 15.5 hours, while the clarified milk reduced it in 17.9 hours.

Marshall and Hood (27) found that "in market milk, the number of organisms is usually increased after clarification, as revealed by the plating method. This is doubtless due to disruption of colonies." They also observed that the clarifier was able to eliminate bacteria in "no small degree, but no differentiation between pathogens and non-pathogens can be made."

In a later article Marshall and Hood (28) report that the clarifier had effected eliminations of from 24 to 99 per cent of bacteria from milk, the large organisms and colonies of organisms being removed most readily. Pure cultures of bacteria were used in this work.

Dahlberg and Marquardt (6), in their report on the effect of clarification on the bacterial content of milk, summarize as follows: "With one



exception the official plate count was decreased in milk with a count below 100,000 per c.c., but there was a tendency to increase the count of milk with more than 100,000 as a result of clarification. A decrease in the size of bacterial clumps in clarified milk of low count was shown by direct microscopic examination."

Traum and Hart (41) found that milk which had been naturally infected with tubercle bacilli, after having been clarified in a large milk plant, caused tuberculosis in guinea pigs which were inoculated with it.

Marpman (26) reports the tubercle organisms to be of about the same specific gravity as normal milk, varying from 1.018 to 1.046.

Moore (33) artificially infected milk with tubercle bacilli from glycerol bouillon and blood serum cultures at the rate of 7 c.c. to 4000 c.c. of milk, and found that after running this through a hand separator the skim milk and cream both caused tuberculosis when inoculated into guinea pigs. He repeated the experiment with other pathogenic organisms and found the bacteria in the skim milk.

#### STATEMENT OF THE PROBLEM

The magnitude of the industry warrants the introduction of improved methods of manufacture, and it was for the purpose of determining the effect of clarification on the quality of cheddar cheese that the work herein reported was undertaken.

The studies were divided into two parts. The first deals with the effect of clarification on the numbers and types of bacteria present in milk and was carried out for the purpose of determining whether or not the effect of clarification on the bacteria in milk is such as to suggest an influence on the quality of cheese obtained from the milk. The second involves the making and study of cheese from unclarified and clarified milk from the same lot.

The work has been conducted for several years. Part of the cheese was made in Utah and part of it in Iowa. Therefore, it has been possible to study the effect of clarification under different conditions.

#### PART I. THE EFFECT OF CLARIFICATION ON THE BACTERIA IN MILK

Since the numbers and types of bacteria present in milk may show considerable variation, an extended study of the effect of clarification on the flora of milk was undertaken. The investigation involved determinations of the effect of clarification on the numbers and types of bacteria in milk using a normal and tenth-normal rate of inflow; these were intended to show whether or not the clarifier had a selective action on the organisms. A study of the types of bacteria in clarifier slime and in the sediment obtained from milk which had been centrifuged in tubes also was included. Because of their use in cheese factories in determining the quality of milk, the methylene blue reduction test and the fermentation test were compared with the plate count from the standpoint of showing the changes caused by clarification. A study was made of the size and specific gravity of the organisms which appeared to be eliminated by the clarifier and an attempt made to classify them.

#### METHODS

The milk used for the bacteriological work was that received by the Dairy Department of the Iowa State College. No attempt was made to

select any of the milk, but in all cases that which was studied represented the general milk supply. Care was taken always to steam all parts of the equipment with which the milk was to come in contact. The milk was well agitated in a 100-gallon coil vat before clarification, and was then pumped through a short line of sanitary pipe to the clarifier. A DeLaval No. 105 clarifier was used throughout the experiments. The clarified milk samples were taken after at least ten gallons of milk had run through the clarifier to reduce all possible contamination from the pump, pipe, or clarifier, and also to be sure that the clarifier was working normally. The samples of milk were transferred to sterile tubes by means of sterile pipettes. Whenever the samples were to be held for some time, they were placed in ice water as soon as collected to prevent multiplication of the bacteria. Various clarification temperatures were used during the earlier part of the investigational work in order to determine at what temperature the clarifier caused the least change in the physical condition of the milk. It was found that when clarifying at temperatures from 15.5° to 37.8°C. (60° to 100°F.) considerable foam, rich in fat, was formed, while at temperatures from 10° to 15.5°C. (50° to 60°F.) the physical state of the milk was not materially influenced. Therefore, it was decided to clarify the milk at the temperatures at which it was received, which were generally about 12.8°C. (55°F.). Ordinarily, the clarifier was operated at a normal capacity, but in a number of trials the rate of inflow was reduced to about one-tenth normal.

In the preparation of the clarifier slime for plating, a representative sample was taken from the clarifier bowl by means of a sterile spatula and placed in a sterile mortar. The slime was thoroughly triturated with sterile water until it formed a homogeneous viscous material. A small amount of this was then transferred to a water blank and plates poured after the proper dilutions had been made.

Beef infusion agar, containing 1.5 per cent shred agar and 0.5 per cent peptone, adjusted to a reaction of plus 1.0 Fuller's scale, was used for all the counts. This medium was chosen because it was desired to use one which would be most favorable for the growth of the bacteria in the milk. Duplicate plates were prepared, which were incubated at 37°C. (98.6°F.) for two days.

In the study of the types of bacteria present in unclarified and clarified milk and in the slime, 50 contiguous colonies from representative areas on the plates were picked into sterile litmus milk. These cultures were kept at room temperature for ten days, and then classified into the following groups: acid coagulators, acid non-coagulators, inert, alkali producers, and neutral, alkali, and acid peptonizers.

The method used for the methylene blue reduction test was that recommended in the Standard Methods of Milk Analysis (40). The samples used for the methylene blue test were also used for the fermentation test; the tubes were kept for six days at room temperature for observations on the character of the fermentations occurring.

#### RESULTS

##### 1. *Influence of Clarification on the Number of Bacteria in Milk*

In the study of the influence of clarification on the number of bacteria in milk, 43 comparisons of unclarified and clarified milk were made, using the plate method. Table 1 gives the bacterial counts obtained and the per-

centage change in numbers due to clarification. The counts on the unclarified milk ranged from 15,300 to 1,240,000 per c.c. Clarification caused an increase in the bacterial count in 20 trials and a decrease in 23. The increases in the counts varied from 3.1 to 143.7 per cent and averaged 54.6 per cent, while the decreases varied from 0.4 to 59.1 per cent and averaged 24.9 per cent. Considering the 43 comparisons, there was an average increase of 12.1 per cent as a result of clarification.

The increases due to clarification as determined by the plate count are only apparent, since there was no appreciable contamination, and are accounted for by the breaking up of bacterial clumps and chains by the clarifier.

TABLE 1. *Influence of Clarification on the Number of Bacteria in Milk.*

Date			Bacteria per c.c.		Percentage	
			Unclarified	Clarified	Increase	Decrease
1926	Nov.	8	19,000	27,000	42.1	
		12	27,500	45,000	63.6	
		15	137,000	136,500		.4
		19	21,500	45,000	109.3	
		22	32,500	22,150		31.8
		29	29,000	54,000	86.2	
	Dec.	11	79,500	82,000	3.1	
		13	88,000	102,000	15.9	
		15	138,500	159,000	14.8	
		14	558,000	532,000		4.6
		18	15,300	27,800	81.6	
		20	66,500	105,000	57.9	
	1927 Jan.	5	199,000	116,000		41.7
		7	297,000	250,000		15.8
		8	53,000	45,000		15.1
		10	134,500	130,500		3.0
		12	45,500	43,000		5.5
		14	21,000	39,500	88.1	
		15	322,000	139,200		56.7
		17	185,000	176,500		4.6
		19	50,500	43,000		14.8
		22	17,200	15,800		8.1
		24	44,000	18,000		59.1
		26	199,000	97,500		51.0
		29	146,000	215,000	47.2	
	Feb.	7	53,500	50,000		6.5
		11	147,000	128,000		12.9
		14	800,000	400,000		50.0
		15	1,240,000	970,000		21.8
		18	78,000	86,000	10.2	
		19	252,000	266,000	5.6	
		22	225,000	339,000	50.7	
		23	390,000	460,000	17.9	
		25	320,000	780,000	143.7	
		26	400,000	290,000		27.5
		28	103,000	149,000	44.7	
	Mar.	2	48,000	63,000	31.3	
		4	75,000	111,000	48.0	
		5	380,000	360,000		5.3
		7	42,000	97,000	130.9	
		11	540,000	305,000		43.5
		12	530,000	330,000		37.7
		14	805,000	370,000		54.0

An analysis of the data given in table 1, shows that 23 of the 43 counts on unclarified milk were higher than 100,000 per c.c., and 20 were lower. With the 23 counts above 100,000 per c.c., clarification caused a decrease in 16, or 70 per cent, and an increase in seven, or 30 per cent, while with the 20 samples of milk showing counts lower than 100,000 per c.c. clarification caused a decrease in 7, or 35 per cent, and an increase in 13, or 65 per cent. This shows that with the milk studied, clarification was more likely to cause a decrease in the count when the milk contained over 100,000 bacteria per c.c. than when it contained a smaller number. Other investigators report similar results, although the available data show considerable variations.

## 2. Influence of Clarification With Normal and With Reduced Rate of Inflow on the Number of Bacteria in Milk.

A comparison was made of the influence of clarification on the number of bacteria in milk, using a normal and a tenth-normal rate of inflow. The plate counts obtained and the percentage change in numbers with 19 lots of milk are shown in table 2.

TABLE 2. Influence of Clarification With Normal and With Reduced Rates of Inflow on the Number of Bacteria in Milk.

Date		Unclarified	Normal inflow		Reduced inflow	
		(Bact. per c.c.)	(Bact. per c.c.)	Pct. decrease	Bact. per c.c.	Pct. decrease
1927	Mar. 26	1,730,000	1,620,000	6.3	1,060,000	38.7
	Apr. 5	2,100,000	1,930,000	8.1	580,000	72.4
	Apr. 6	1,520,000	1,090,000	28.3	360,000	76.3
	Apr. 7	59,000	57,000	3.4	20,500	65.2
	Apr. 8	64,000	52,000	18.7	16,000	75.0
	Apr. 9	46,500	41,500	10.8	16,500	64.5
	Apr. 11	170,000	180,000	—5.9	82,000	51.7
	Apr. 12	230,500	343,000	—48.8	128,000	44.4
	Apr. 13	1,850,000	1,070,000	42.1	417,000	77.4
	Apr. 14	27,000	44,500	—64.8	17,000	37.0
	Apr. 15	90,000	79,000	12.2	47,000	47.3
	Apr. 16	1,220,000	1,600,000	—31.1	710,000	41.8
	Apr. 28	770,000	960,000	—24.7	570,000	25.9
	Apr. 29	3,710,000	2,920,000	21.2	1,670,000	55.0
	May 2	5,310,000	2,110,000	60.2	2,040,000	61.5
	May 3	2,230,000	2,010,000	9.9	435,000	80.5
	May 4	1,220,000	855,000	29.9	595,000	51.1
	May 5	83,500	113,000	—35.3	52,000	37.7
	May 7	600,000	485,000	19.1	320,000	46.7

The counts on the unclarified milk ranged from 27,000 to 5,310,000 per c.c. When the rate of inflow was normal, clarification caused an increase in the count in six trials, and a decrease in 13, but when the rate of inflow was reduced, clarification always caused a decrease. With the normal rate of inflow there was a minimum increase in the counts of 5.9 per cent, a maximum of 64.8, and an average of 35.1, while the minimum decrease was 3.4 per cent, the maximum 60.2, and the average 20.8; considering the 19 comparisons there was an average decrease of 3.1 per cent. When the rate of inflow was reduced there was a minimum decrease of 25.9 per cent, a maximum of 80.5, and an average of 55.3. These data show that by decreasing the rate of inflow it was possible to remove, on an average, more than half of the bacteria present in milk.



Thirteen of the 19 samples of milk contained more than 100,000 bacteria per c.c., while six had less. With the 13 counts above 100,000 per c.c. clarification with the normal rate of inflow caused a decrease in nine, or 70 per cent, and an increase in four, or 30 per cent, while with the six counts below 100,000 per c.c. there was a decrease in four or 67 per cent, and an increase in two, or 33 per cent. The milk used for these trials contained on an average more bacteria than did the milk used for the trials reported in table 1. However, the results obtained in both series of determinations with the milk containing more than 100,000 bacteria per c.c. check very closely.

TABLE 3. *Types of Bacteria Present in Uncolified and Clarified Milk.*

Date		Percentage								
		Acid		Inert	Alkali	Peptonizers				
		Coagu- lators	Non-coagu- lators			Neutral	Acid	Alkali		
1926 Nov.	8	Uncl.	49.1	16.3	16.3	14.3	2.0	2.0	0	
		Cl.	83.4	9.3	5.4	1.9	0	0	0	
	12	Uncl.	10.9	60.9	15.2	2.2	4.3	6.5	0	
		Cl.	10.6	61.7	19.1	4.3	0	4.3	0	
	15	Uncl.	29.6	37.0	12.9	13.0	0	5.6	1.9	
		Cl.	40.4	42.4	1.9	11.5	0	1.9	1.9	
	19	Uncl.	43.7	23.0	16.6	14.6	0	0	2.1	
		Cl.	20.0	44.0	28.0	8.0	0	0	0	
	22	Uncl.	10.0	52.0	10.0	24.0	0	0	4.0	
		Cl.	10.0	50.0	18.0	20.0	0	0	2.0	
	Dec.	6	Uncl.	53.8	23.1	0	15.4	0	7.7	0
			Cl.	39.6	30.1	7.7	11.3	1.9	9.4	0
Nov.	29	Uncl.	30.0	18.0	10.0	40.0	0	2.0	0	
		Cl.	24.0	22.0	10.0	38.0	0	6.0	0	
Dec.	11	Uncl.	42.0	14.0	14.0	22.0	0	8.0	0	
		Cl.	44.0	38.0	0	14.0	0	4.0	0	
	13	Uncl.	14.5	12.3	10.2	59.0	2.0	2.0	0	
		Cl.	12.0	22.0	4.0	60.0	0	0	2.0	
	3	Uncl.	86.0	10.0	0	4.0	0	0	0	
		Cl.	85.7	8.2	2.1	2.0	0	2.0	0	
	4	Uncl.	86.0	10.0	0	4.0	0	0	0	
		Cl.	96.0	0	0	4.0	0	0	0	
	18	Uncl.	10.2	40.8	22.5	26.5	0	0	0	
		Cl.	10.0	40.0	20.0	30.0	0	0	0	
	20	Uncl.	22.0	24.0	8.0	40.0	0	6.0	0	
		Cl.	52.0	22.0	4.0	18.0	0	4.0	0	
1927 Jan.	5	Uncl.	24.0	16.0	8.0	32.0	0	20.0	0	
		Cl.	20.0	34.0	2.0	36.0	0	8.0	0	
	7	Uncl.	7.8	7.8	2.0	39.2	0	43.2	0	
		Cl.	10.0	22.0	0	38.0	0	30.0	0	
	8	Uncl.	13.7	31.4	19.6	5.9	0	29.4	0	
		Cl.	14.0	40.0	18.0	6.0	2.0	20.0	0	
	10	Uncl.	4.0	22.0	6.0	50.0	0	18.0	0	
		Cl.	0	28.0	4.0	50.0	2.0	16.0	0	
	12	Uncl.	16.0	20.0	2.0	24.0	2.0	36.0	0	
		Cl.	18.0	20.0	4.0	40.0	4.0	14.0	0	
	14	Uncl.	8.0	36.0	12.0	32.0	2.0	10.0	0	
		Cl.	16.0	40.0	12.0	24.0	0	8.0	0	
17	Uncl.	24.0	24.0	16.0	18.0	0	18.0	0		
	Cl.	30.0	22.0	10.0	28.0	0	10.0	0		
Feb.	14	Uncl.	36.0	36.0	4.0	16.0	0	8.0	0	
		Cl.	56.0	20.0	10.0	10.0	4.0	0	0	

3. *The Effect of Clarification on the Types of Bacteria Present in Milk.*

The results of a study of the types of bacteria present in 21 lots of milk before and after clarification are reported on a percentage basis in table 3. The data show that the acid forming groups predominated in both the unclarified and clarified milk, while the neutral and acid peptonizers were the least numerous. Sometimes the acid coagulators were present in larger numbers than the acid non-coagulators, and sometimes the reverse was true. Separation of the acid formers into two groups on the basis of whether or not coagulation occurs is of questionable importance since with certain of the *Streptococcus lactis* organisms (19) the rate of coagulation of litmus milk is a character which may undergo definite fluctuations. In a few comparisons the alkali formers were present in large numbers in the unclarified milk, but they were present also in considerable numbers in the milk after clarification. Whenever a certain group of organisms was present in the unclarified milk, it was present nearly always in the clarified milk, although there was usually a change in the percentage. The results show that clarification caused increases and decreases in the per-

TABLE 4. *The Types of Bacteria Present in Unclarified Milk and in the Slime Obtained from It.*

Date		Percentage						
		Acid		Inert	Alkali	Peptonizers		
		Coagu- lators	Non-coagu- lators			Neutral	Acid	Alkali
1926 Dec. 11	U.*	42.0	14.0	14.0	22.0	0	8.0	0
	Sl.**	41.4	12.0	0	46.6	0	0	0
	U.	10.2	40.8	22.5	26.5	0	0	0
	Sl.	3.9	11.7	21.6	62.8	0	0	0
1927 Jan. 5	U.	24.0	16.0	8.0	32.0	0	20.0	0
	Sl.	22.7	0	0	75.0	0	0	2.3
7	U.	7.8	7.8	2.0	39.2	0	43.2	0
	Sl.	20.0	14.0	0	64.0	0	2.0	0
8	U.	13.7	31.4	19.6	5.9	0	29.4	0
	Sl.	3.9	23.5	31.4	19.6	0	21.6	0
12	U.	16.0	20.0	2.0	24.0	2.0	36.0	0
	Sl.	4.0	14.0	0	68.0	2.0	12.0	0
14	U.	8.0	36.0	12.0	32.0	2.0	10.0	0
	Sl.	28.0	16.0	10.0	32.0	0	14.0	0
15	U.	68.6	15.7	3.9	5.9	0	5.9	0
	Sl.	20.0	2.0	0	74.0	0	4.0	0
17	U.	24.0	24.0	16.0	18.0	0	18.0	0
	Sl.	58.3	0.0	2.0	35.4	0	4.3	0
19	U.	4.0	6.0	22.0	30.0	2.0	36.0	0
	Sl.	8.0	2.0	2.0	82.0	0	6.0	0
22	U.	34.0	26.0	4.0	26.0	0	10.0	0
	Sl.	12.0	58.0	2.0	22.0	0	6.0	0
24	U.	14.0	14.0	4.0	54.0	0	14.0	0
	Sl.	46.0	14.0	0	40.0	0	0	0
26	U.	42.0	2.0	0	54.0	0	2.0	0
	Sl.	26.0	72.0	0	0	0	0	2.0
29	U.	52.0	20.0	5.0	13.0	0	10.0	0
	Sl.	57.4	0	0	12.8	0	29.8	0
Feb. 7	U.	53.0	13.7	11.8	17.6	0	3.9	0
	Sl.	26.0	6.0	2.0	64.0	0	2.0	0
11	U.	90.0	2.0	0	8.0	0	0	0
	Sl.	76.0	8.0	2.0	14.0	0	0	0

TABLE 4. *Continued.*

Date		Percentage						
		Acid		Inert	Alkali	Peptonizers		
		Coagu- lators	Non-coagu- lators			Neutral	Acid	Alkali
1926 Feb. 12	U.	98.0	2.0	0	0	0	0	0
	Sl.	98.0	0	0	2.0	0	0	0
14	U.	36.0	36.0	4.0	16.0	0	8.0	0
	Sl.	26.0	26.0	2.0	44.0	0	2.0	0
15	U.	82.0	16.0	0	2.0	0	0	0
	Sl.	42.0	12.0	6.0	26.0	0	14.0	0
18	U.	82.0	2.0	0	12.0	0	4.0	0
	Sl.	40.0	2.0	4.0	54.0	0	0	0
19	U.	74.0	12.0	0	8.0	0	6.0	0
	Sl.	90.0	4.0	0	4.0	0	2.0	0
22	U.	10.0	56.0	0	24.0	0	10.0	0
	Sl.	10.0	60.0	0	26.0	0	4.0	0
23	U.	90.0	0	6.0	2.0	0	2.0	0
	Sl.	84.0	0	2.0	10.0	0	4.0	0
25	U.	88.0	0	2.0	10.0	0	0	0
	Sl.	88.0	0	0	12.0	0	0	0
26	U.	100.0	0	0	0	0	0	0
	Sl.	64.0	12.0	10.0	8.0	0	6.0	0
28	U.	72.0	16.0	8.0	4.0	0	0	0
	Sl.	30.0	16.0	16.0	24.0	0	14.0	0
Mar. 2	U.	12.0	82.0	6.0	0	0	0	0
	Sl.	34.0	20.0	10.0	30.0	0	6.0	0
4	U.	50.0	40.0	0	4.0	0	6.0	0
	Sl.	38.0	24.0	6.0	24.0	0	8.0	0
5	U.	90.0	10.0	0	0	0	0	0
	Sl.	74.0	10.0	2.0	10.0	2.0	2.0	0
7	U.	32.0	52.0	4.0	2.0	0	8.0	2.0
	Sl.	26.0	68.0	2.0	2.0	0	2.0	0
11	U.	46.0	20.0	2.0	20.0	8.0	4.0	0
	Sl.	32.0	20.0	4.0	38.0	2.0	4.0	0
12	U.	22.0	8.0	4.0	34.0	4.0	28.0	0
	Sl.	2.0	4.0	0	80.0	4.0	10.0	0
14	U.	66.0	10.0	4.0	10.0	10.0	0	0
	Sl.	34.0	52.0	8.0	4.0	2.0	0	0

\*Unclarified.

\*\*Slime.

centages of the various groups, but did not cause a consistent elimination of any single group.

When one considers that milk consists of various complex substances and that the bacteria present may show daily variations both in numbers and in types, it would be expected that the effect of clarification would be variable. The changes caused by clarification were not significant enough to warrant any conclusions regarding a selective action of the clarifier.

An analysis of the data given in table 3 shows that clarification caused increases in the groups approximately as often as decreases, with the exception of the acid peptonizers, which were decreased in 82.4 per cent of the comparisons in which they were present in the original milk, but since these organisms were present in the milk in comparatively small numbers, the significance of this is not great. On the average, the acid coagulators were increased, and the alkali formers decreased by clarification. This is of particular interest because of later studies on the types of bacteria present in clarifier slime.

#### 4. *The Types of Bacteria Present in Unclarified Milk and in the Slime Obtained from It.*

Although the results obtained in section 3 did not indicate any selective action by the clarifier on the bacteria present in milk, it was decided to study this further under conditions favorable for determining such an effect. A study of the types of bacteria present in unclarified milk and in the slime obtained from it was made, because if the clarifier has any selective influence on the bacteria in the milk, this should be quite evident from the types of bacteria present in the slime. The results of 33 comparisons are reported on a percentage basis in table 4. The data show that the types of bacteria present in the milk, in general, were the same as those present in the slime. The types of bacteria present in the milk were similar to those present in the unclarified milk studied in section 3. The acid coagulators usually predominated, but sometimes the acid non-coagulators were the most numerous; the neutral and alkali peptonizers were the least prominent. In the slime the acid formers usually predominated, although occasionally the alkali formers were the most prominent, while the neutral and alkali peptonizers were present in the smallest percentages. Wide variations were noted in the percentages of acid coagulators and alkali formers present, but no regular elimination of either of these two groups from the milk seems to have taken place.

A comparison of the differences in the types of bacteria present in the milk and in the slime is shown in table 5, together with a summary of these differences. The only groups which showed a fairly consistent change as a result of clarification were the acid coagulators and the alkali formers. The average changes were 8.7 per cent decrease in the acid formers and a 16.8 per cent increase in the alkali formers in the slim as compared with those present in the milk. Whether these changes were actually brought about by the clarifier is difficult to say. As has already been pointed out, clarification slightly increased the percentage of acid coagulators in the milk and decreased the percentage of alkali formers. This would suggest that there should be a corresponding decrease and increase in the same groups in the slime, and the figures shown in table 5 indicate that such a change did take place. The summary of the changes shows that an increase and a decrease occurred in the various groups of organisms, the most consistent decrease occurring in the acid forming and the most consistent increase in the alkali forming groups.

The average change of the acid coagulators is about three times and that of the alkali formers about twenty times as great as the probable error of the mean, while the average changes of the other groups are in the same order of magnitude as the probable error of the mean. This would indicate that the change in the alkali-forming group had considerable significance and the change in the acid coagulating group some significance. From the theory of probability the chances are slight that the changes in these two groups were due to experimental error.

Microscopic examinations of the slime showed the presence of many gram-positive clumps and chains of bacteria of varying size, as well as numerous gram-positive and gram-negative organisms, isolated and in pairs. Large gram-negative rods were abundant; they were usually pres-



TABLE 5. Differences in the Types of Bacteria Present in Unclarified Milk and in the Slime Obtained from It.

Date	Changes in the types of organisms in the slime as compared with the types present in unclarified milk										Alk. pept.	
	Acid coagulators		Acid non-coagulators		Inert		Alkali		Neut. pept.		Acid pept.	
	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease
1926 Dec. 11	0.6		2.0		14.0		24.6				8.0	
18	6.3		29.1				36.3					
1927 Jan. 5	1.3		16.0		8.0		43.0				20.0	
7	12.2		6.2				24.8				41.2	
8	9.8		7.9		11.8		13.7				7.8	
12	12.0		6.0		2.0		44.0		0		24.0	
14	20.0		20.0		2.0		0		0	4.0		
15			13.7		3.9		68.1		2.0		1.9	
17	34.3		24.0		14.0		17.4		2.0		13.7	
19	4.0		4.0		20.0		52.0				30.0	
22	22.0		32.0		2.0		4.0				4.0	
24	32.0		0		4.0		14.0				14.0	
26	16.0		70.0				54.0				2.0	
29	5.4		20.0		5.0		0.2			19.8		2.0
Feb. 7	27.0		7.7		9.8		46.4				1.9	
11	14.0		6.0		2.0		6.0					
12	0		2.0				2.0					
14	10.0		10.0		2.0		28.0			6.0		
15	40.0		4.0		6.0		24.0			14.0		
18	42.0		0		4.0		42.0			4.0		
19	16.0		8.0							4.0		
22	0		4.0		4.0		2.0			2.0		
23	6.0				2.0		8.0			6.0		
25	0						2.0			14.0		
26	36.0		12.0		10.0		8.0			2.0		
28	42.0		0		8.0		20.0			6.0		
Mar. 2	22.0		62.0		4.0		30.0			6.0		
4	12.0		16.0		6.0		20.0			2.0		
5	16.0		0		2.0		10.0		2.0	2.0		
7	6.0		16.0		2.0		0		0	6.0		
11	14.0		0		2.0		18.0		6.0	0		2.0
12	20.0		4.0		4.0		46.0		0	0		
14	32.0		42.0		4.0		6.0		8.0	18.0		
Av. change	8.7		2.2		1.4		16.8		2.3	5.1	0.7	
	$\sigma = 26.9$	$\sigma = 22.6$	$\sigma = 22.6$	$\sigma = 7.55$	$\sigma = 7.16$	$\sigma = 5.65$	$\sigma = 18.21$	$\sigma = 1.96$				
	p.e. = 17.8	p.e. = 15.0	p.e. = 4.95	p.e. = 4.8	p.e. = 3.78	p.e. = 1.44	p.e. = 2.56	p.e. = 1.31				
	p.e.m. = 3.16	p.e.m. = 2.69	p.e.m. = 0.947	p.e.m. = 0.846	p.e.m. = 1.44	p.e.m. = 2.56	p.e.m. = 0.76					

TABLE 5. *Continued.*

Summary of the changes in the various groups						
	Increase		Decrease		No change	
	Times	Per cent	Times	Per cent	Times	Per cent
Acid coagulators	8	24.2	22	66.7	3	9.1
Acid non-coag.	8	25.8	18	58.1	5	16.1
Inert	11	37.9	18	62.1	0	0
Alkali	25	75.7	6	18.2	2	6.1
Neut. Pept.	1	14.3	4	57.1	2	28.6
Acid Pept.	9	32.1	18	64.3	1	3.6
Alkali Pept.	2	66.7	1	33.3	0	0

ent in pairs, and corresponded in morphology and staining reaction to those of the alkali formers which had been isolated from plates.

The changes in the percentages of acid and alkali formers may have been due to a selective action of the clarifier. They may also have been due to a breaking-up of bacterial clumps and chains by the clarifier, while the increase in the percentage of alkali formers may be accounted for by assuming that these organisms were associated with the cells and dirt particles in the milk, and since these, for the most part, were removed in the slime, the organisms would be removed with them. However, this latter explanation is not entirely logical, because if the alkali formers were held by the cells and dirt they should practically all have been removed, while the data show that on an average the slime contained only 16.8 per cent more of these organisms than the unclarified milk.

The results obtained in this study seem to justify the conclusion, that if the clarifier had a selective action on the bacteria present in the milk used, the alkali formers were the ones responding to this influence.

##### 5. *The Effect of Clarification With Normal and Reduced Rate of Inflow on the Types of Bacteria Present in Milk.*

In order to study the effect of prolonged exposure of milk to centrifugal force in a clarifier on the types of bacteria present, a series of 18 comparisons was made in which a normal and a one-tenth normal rate of inflow were used. The primary object was to see if it was possible, by means of a greater clarifying efficiency, to cause a significant change in the flora of the milk. The data obtained showed that the acid formers predominated in both the unclarified milk and in the milk clarified with either method of clarification while the neutral and alkali peptonizers were the least numerous.

The results agree with those obtained in section 3. The types of bacteria present in the clarified milk were similar to those in the unclarified milk. Although there were slight variations in the types present, no great elimination of any of the groups took place.

##### 6. *The Effect of Clarification With Normal and Reduced Rate of Inflow on the Bacteria in Milk, as Determined by the Plate Count, the Methylene Blue Test and the Fermentation Test.*

A series of determinations of the effect of clarification on the bacteria in milk with a normal and with a reduced rate of inflow was made, using the plate count, the methylene blue test, and the fermentation test. The data obtained in nine comparisons are shown in table 6.

TABLE 6. *The Effect of Clarification with Normal and Reduced Rate of Inflow on the Bacteria in Milk, as Determined by the Plate Count, the Methylene Blue Test, and the Fermentation Test.*

## Plate count

Date			Unclarified (bacteria per c.c.)	Clarified			
				Normal inflow (bacteria per c.c.)	Percentage decrease	Reduced In- flow (bacteria per c.c.)	Percentage decrease
1927 May	25		700,000	470,000	32.9	140,000	80.0
	26		1,035,000	745,000	28.0	560,000	45.9
June	20		3,310,000	1,980,000	40.2	550,000	83.4
	21		7,530,000	6,600,000	12.3	700,000	12.3
	22		2,780,000	1,890,000	32.0	1,090,000	60.8
July	6		615,000	475,000	22.8	350,000	43.1
	7		2,820,000	2,580,000	8.5	1,060,000	62.4
	8		750,000	580,000	22.6	330,000	56.0
	9		920,000	750,000	18.5	270,000	70.7
			Av. 24.2				Av. 57.2

## Methylene blue test

Date			Unclarified (minutes to reduce)	Clarified			
				Normal inflow (minutes to reduce)	Increase in re- duction time due to clarifi- cation (min.)	Reduced in- flow (minutes to reduce)	Increase in re- duction time due to clarifi- cation (min.)
1927 May	25		135	180	45	210	75
	26		235	255	20	285	50
June	20		90	105	15	135	45
	21		60	90	30	120	60
	22		165	210	45	240	75
July	6		240	240	0	270	30
	7		120	140	20	180	60
	8		210	210	0	210	0
	9		210	225	15	240	30
			Av. 21				Av. 47

## Fermentation test

Date			Unclarified	Clarified (character of curd)	
				Normal inflow	Reduced inflow
1927 May	25		Sl. liq. near sur- face of curd	Solid curd	Sl. liq. near sur- face of curd
	26		Solid curd	Solid curd	Solid curd
June	20		Sl. gassy	Sl. gassy	Sl. gassy
	21		Solid curd	Solid curd	Solid curd
	22		Gassy	Gassy	Gassy
July	6		Badly gassy	Badly gassy	Badly gassy
	7		Gassy	Sl. gassy	Gassy
	8		Gassy	Gassy	Gassy
	9		Sl. gassy	Sl. gassy	Sl. gassy

The bacterial counts were quite high, on an average. Clarification always resulted in a decrease in the counts. There was an average decrease of 24.2 per cent when a normal rate of inflow was used, and an average decrease of 57.2 per cent when a one-tenth normal rate of inflow was used. The reduction time of the unclarified milk ranged from 60 to 240 minutes and averaged 163 minutes, that of the normally clarified milk ranged from

90 to 255 minutes and averaged 184 minutes, while that of milk clarified with a reduced rate of inflow ranged from 120 to 285 minutes and averaged 210 minutes. With the normal rate of inflow, clarification caused an increase in the reduction time in seven trials and no change in two and with the reduced rate of inflow it caused an increase in eight trials and no change in one. The greatest increase in the time with the normal inflow was 45 minutes and with the reduced inflow 75 minutes. The average increases when all comparisons are considered were 21 minutes with normal clarification and 47 minutes with clarification involving a reduced rate of inflow.

The data would indicate that clarification with both rates of inflow resulted in most cases in a noticeable increase in the reduction time. There seems to be no definite correlation, however, between the counts of the unclarified or clarified milk and the reduction time when the counts for the different runs are considered. Since clarification always resulted in a decrease in the counts in this particular series, these data are therefore not applicable to runs where clarification results in an increase.

The results obtained by the methylene blue test agreed, in general, with those obtained by the plate method. It would seem, however, that the plate method was the more refined in showing the differences in the numbers of bacteria of milk caused by clarification.

The reason why the methylene blue test does not show relatively small differences in the numbers of bacteria in milk, may be accounted for by the differences in the ability of various types of bacteria to decolorize methylene blue and to the variations in their development.

The observations of the fermentation test would indicate that clarification had no influence on the type of fermentation taking place, whether a normal or a reduced rate of inflow was used. The character of the curd which formed from the clarified milk, in all cases, was similar to that which formed from the unclarified milk.

#### *7. Comparison of the Effect of Centrifugal Force on the Numbers and Types of Organisms Present in Uncentrifuged Milk, and in the Sediment Obtained in Centrifuge Tubes.*

In order to obtain some additional data on the effect of centrifugal force on milk, tests were made in which 15 c.c. samples of uncentrifuged milk were placed in conical tubes and centrifuged at room temperature for varying periods of time; the speed was 1900 revolutions per minute and the circle through which the outer edge of the tubes passed was 15 inches. The sediment was then diluted to 15 c.c. with sterile water and the numbers and types of organisms determined.

The data obtained showed that in a series of four comparisons, after five minutes centrifuging, a minimum of 5.8, a maximum of 20.2, and an average of 12.6 per cent of the total numbers of bacteria present in the milk were in the sediment, while after the milk had been exposed to centrifugal force for 20 minutes a minimum of 9.5, a maximum of 29.9, and an average of 16.7 per cent were in the slime.

The results of a study of the types of bacteria present in uncentrifuged milk and in the sediment obtained from it after centrifuging samples of milk of the same lot for 5, 20 and 30 minutes, respectively, in four com-



parisons, and for 5 and 20 minutes in four other comparisons showed that the acid formers were usually the most numerous types in both the milk and in the sediment, but sometimes the alkali formers or acid peptonizers were the most prominent. Centrifuging caused some slight variations in the types, but whenever a certain type was present in the milk it was usually found in the sediment. Even prolonged exposure of the milk to centrifugal force did not consistently cause a complete elimination of any of the types present in the milk.

It would seem that the exposure of milk to centrifugal force in tubes, even for a considerable time, did not materially affect the flora of the milk.

Although centrifuging of the naturally infected milk did not cause much change in the types of organisms present, it was decided to study the effect of centrifugal force on samples of low count milk heavily inoculated with large alkali forming bacteria or with yeasts obtained from agar slope cultures. Raw milk was used in preference to sterile milk because in the latter some of the constituents are changed physically and chemically by the heating process. In all cases, both with the alkali formers and with the yeasts, centrifuging for one-half hour caused a decrease in the numbers present. The number of organisms present in the milk and cream layer after these had been mixed, when added to the numbers in the sediment, should equal the total number of organisms present in the milk before centrifuging. This, as a rule, did not occur, probably due to a clumping of the organisms in the sediment, since microscopic examination of the sediment showed the presence of large numbers of clumps of the inoculated organisms.

#### 8. *The Specific Gravity of Yeasts and Alkali Forming Bacteria.*

An attempt was made to determine the specific gravity of some of the alkali producing organisms isolated because of their relative abundance in the slime. The specific gravity of a still larger organism, *Torula cremoris*, also was determined.

The alkali formers were grown in Petri dishes on beef infusion agar, while beef extract agar containing lactose was used for the yeast. Usually the growth obtained from eight or ten dishes was employed in each determination. As soon as a good growth had occurred this was carefully scraped off with a sharp steel blade and transferred quickly to a tared, dry, 25 c.c. pycnometer. Whenever the growth was sufficient, duplicate determinations were made. The specific gravity was determined by comparing the weight of a certain volume of the organisms with the weight of an equal volume of distilled water at the same temperature. In making the determinations, four different weighings were made, as follows: the dry pycnometer, the pycnometer plus the bacterial growth, the pycnometer filled with distilled water, and the pycnometer filled with the bacterial growth and distilled water. The specific gravity of the bacterial growth was obtained by dividing the weight of this as determined by subtraction by the weight of the volume of water which it displaced; the latter represented the difference between the weight of the water containing organisms and the weight of distilled water necessary to fill the pycnometer. In two determinations plain bouillon was used as a medium for the alkali formers. Five hundred c.c. lots of this were inoculated, and when a good growth had occurred

they were centrifuged in 50 c.c. tubes; the sediment obtained was then placed in pycnometers and the specific gravity determined.

With *Torula cremoris* the values obtained ranged from 1.037 to 1.102, with an average of 1.079. With the alkali formers, when agar was the medium, the values ranged from 1.029 to 1.123, with an average of 1.0497, but when bouillon was used the specific gravities were 1.025 and 1.032, with an average of 1.0285.

The duplicate determinations sometimes showed variation. The smallest difference between checks was 0.003, the largest 0.019, and the average of the seven runs where checks were made was 0.009. The growth obtained from ten plates usually weighed about one gram, but even with heavy growth the amount obtained was too small for accurate determinations.

The results, of course, cannot be directly applied to milk because of the variations in the composition and size of bacteria grown in different kinds of media. If the specific gravities of the alkali formers, as they occur in milk, are similar to those obtained when they are grown on agar and in bouillon, it would seem that it would be difficult, considering the short exposure to the centrifugal force, for the clarifier to remove a large percentage of them.

#### 9. *Comparison of Morphological, Cultural and Biochemical Characteristics of Alkali Forming Bacteria.*

The relative abundance of the alkali forming bacteria in the clarifier slime suggested the isolation and study of organisms of this type. The arbitrary grouping of these organisms on the basis of their ability to produce alkali in milk does not indicate that they possess other characteristics in common, and for this reason a more detailed study of these bacteria would be expected to lead to a division of them into groups or types. From several hundred litmus milk cultures of alkali forming bacteria obtained in the investigation of the types of bacteria present in milk and in clarifier slime, 31 were selected. After having been purified by plating and pickling, the organisms were studied on the basis of morphology, staining reaction, motility, growth in or on various media, gelatin liquefaction, reaction changes in media, and the production of indol and nitrite.

The microscopic examination of the 31 cultures showed that when the organisms were grown in litmus milk at either room temperature or 37°C. (98.6°F.) they were medium to large, plump, rod-shaped, non-spore forming, gram-negative organisms, having rounded ends and arranged in pairs, but when they were grown on infusion agar at both the above temperatures the cells were much smaller and almost spherical. Motility was regularly noted. Growth always occurred near the surface when milk or bouillon was used as a medium and only on the surface with agar stabs. At room temperature and at 37°C. (98.6°F.) the growth on 24-hour agar slopes was moderate, white, filiform, glistening, and slightly raised, while after two weeks the growth was abundant, grey-white, filiform, and in most cases raised. A yellowish-brown growth occurred on potato slants. None of the organisms liquefied gelatin. When grown in plain bouillon or bouillon containing glycerol, dextrose, galactose, levulose, lactose, sucrose, maltose, mannitol, or raffinose (reaction plus 0.05 Fuller's scale), an alkaline reac-

tion was always produced after one week as shown by the change in color which appeared on the addition of a few drops of brom-thymol blue indicator to each tube. Indol was not produced in any of the cultures. Two cultures showed nitrite production.

It would seem from the study of these cultures of alkali formers, that, in general, their main characteristics were similar; they were probably varieties of one species of organisms. Therefore, a separation of them into groups was not made.

## PART II. THE INFLUENCE OF CLARIFICATION ON THE QUALITY OF CHEESE

The study of the effect of clarification on the quality of cheese involved the use of 23,000 pounds of milk, representing both unclarified and clarified milk from 67 different lots. Additional trials were carried out in which cheese made from unclarified milk was compared with that obtained from clarified milk to which clarifier slime or pure cultures of alkali forming bacteria had been added. A total of 145 batches of cheese was made, of which 48 were made in Utah and 97 in Iowa.

In a few comparisons the bacterial flora of the ripened cheese was studied for the purpose of determining whether or not it had been modified by clarification.

### HISTORICAL

Up to the present time clarification of milk for cheddar cheese has not been generally employed by cheese factories in the United States. However, the process is being used to some extent in the manufacture of Swiss cheese.

Matheson (29) found that centrifuging the milk resulted in an increase in the higher grades of Swiss cheese. The cheese made from centrifuged milk did not have a flavor different than that made from untreated milk, but in most cases the body was firmer, and there were larger and fewer eyes. He states that this improvement seems to be due to the removal of dirt and cellular elements from the milk.

Hardell (20) reports that in Ohio every factory using pure cultures was clarifying milk for Swiss cheese during the winter of 1926-27. He states that clarification of milk has been an important factor in the improvement of Ohio Swiss cheese. It has resulted in a decrease in the number of eyes, and at the same time it has increased the size of the eyes. The body and texture of the cheese likewise seemed to have been improved. This investigator thinks that the results obtained by the factories substantiate the work of the Bureau of Dairying, United States Department of Agriculture, which consistently demonstrated the value of clarifying milk for Swiss cheese manufacture.

As early as 1894, Babcock (2) started experiments to study the influence of cleaning milk with a centrifugal cream separator on the quality of cheese. He reasoned that, because of the offensive character of the material which accumulated on the inside of the separator bowl, the removal of this from the milk would result in an improvement in the flavor and keeping quality of any milk. Nearly 100 cheese were made by students attending the Wisconsin Dairy School from milk cleaned by a separator, the skim milk and cream being mixed as it came from the machine, and "without exception the flavor and keeping quality of the cheese has been improved."



It was also noticed that cleaning the milk in this way either overcame gassy or "pinholey" curds, or greatly minimized the defect. Babcock thought that cleaning milk with a separator would greatly improve the quality of cheese made from tainted milk. He was uncertain whether the improvement was caused by the aeration to which the milk was subjected when it was passing through the separator, or whether it was due to the removal of the slime. In studying this problem, he found that untreated milk caused gassy cheese, while milk which had been cleaned by running it through a separator, and milk which had been cleaned, but to which separator slime had been added, did not result in gassy cheese. This work was done during the winter season, and when it was repeated during the following summer it was found that "there were just as many pinholes in the curds from the cleaned milk as from that not treated." Babcock summarizes by stating that "although cleaning milk with a separator has not accomplished all that we hoped in the treatment of milk for cheese, we feel that it has been of great benefit, as it has, in nearly every case, improved the quality of the cheese, and the improvement has been more marked with tainted milk than with those in good condition. Especially has it been of benefit for long keeping cheese, as such have retained their flavor much better when made from separator cleaned milk."

Fisk and Price (14) report the results of comparisons of cheddar cheese made from unclarified and clarified milk. The work was carried out during 1919 and 1920 under various conditions in different localities. A total of 82 cheese was made. There was a difference in the average score in favor of the clarified milk cheese of 1.282 for flavor, 0.673 for body and texture, and 1.865 in the total score. "The clarified milk cheese had a firmer, more springy feeling, and showed less of a tendency to puff up than the cheese made from unclarified milk." There appeared to be an improvement in the cheese made when either good or poor quality milk was used, and both with and without starter. The authors state that "the clarifier will sometimes overcome the gas in the milk and curd; at other times it will not overcome this gas, but will change it."

In studies on the effect of clarification on the quality of cheese obtained where low quality and where high quality milk was used, Combs, Martin and Hugglar (5) found that the average total score of cheese made from poor quality milk handled under ordinary conditions was 0.79 points lower for the clarified milk cheese than for the unclarified, whereas when poor quality milk was handled under sanitary conditions, the average total score of the clarified milk cheese was 1.59 points higher than that for the unclarified. When high grade milk handled under ordinary conditions was used for cheese, there was a difference of 2.53 points in the average total score in favor of the clarified milk cheese, and when sanitary conditions were used a difference in favor of the clarified milk cheese of 2.83 points in the average total score. When cheese was made under practical conditions, the average score for flavor was 1.0 points higher for the clarified milk cheese than for the unclarified, and the average score for body and texture 1.16 points higher for the clarified milk cheese than for the unclarified. When the cheese was made under extreme sanitary conditions the average score for flavor was 0.58 points higher for the clarified milk cheese than for the unclarified, and the average score for body and texture



1.66 higher for the clarified milk cheese than the unclarified. The authors conclude that although there was a difference of 1.03 in the average total score in the 41 comparisons in favor of clarification, the clarified milk cheese, under present market conditions, would not sell for a higher price; it would therefore be doubtful whether the process of clarification would be justified in the average cheese factory.

The results obtained by the majority of the many research workers who have studied cheese ripening since Duclaux (9) in 1878 first began this type of investigation, seem to point to four outstanding facts: (1) Acid is necessary for the breaking down of the insoluble calcium paracaseinate to monocalcium paracaseinate, which in turn changes to other forms, (2) bacteria are essential in the production of flavor and aroma and in the development of the desirable body and texture of cheese, (3) an enzyme present in rennet effects an increase in the soluble nitrogen compounds in cheese during ripening, thus aiding in producing a more plastic body, and (4) galactase breaks down the calcium paracaseinate to more soluble products. A large amount of research work has been done in various parts of the world on the subject of cheddar cheese ripening, and many theories have been put forth regarding the changes which take place during the curing process.

Evans, Hastings and Hart (13) in 1914 reported the results of a study of the bacteria concerned in the production of the characteristic flavor of cheese of the cheddar type. They summarize their findings by stating that there are four groups of bacteria present in cheddar cheese in such numbers as to indicate that they must function in the ripening process. They are: (1) *Bacterium lactis acidii*, (2) the *B. casei*, (3) *Streptococcus*, and (4) *Micrococcus*. On the basis of the fermentation powers, each of the four groups may be divided into a number of varieties. The flora of raw milk cheese is varied and includes all the varieties into which the four groups may be divided.

The literature on the subject of cheese ripening consists of several hundred references. Hucker (23) has recently made a review of the bacteriological aspects of cheese ripening and summarizes this by the following statement: "As it stands today, the investigations have clearly demonstrated that the breaking down of the insoluble casein compounds is due to enzymes, either natural or bacterial; while characteristic flavors are produced by the action of certain groups of bacteria (*Bact. casei* or coccus group), which depend upon the products of *B. lactis acidii* present in large numbers during the manufacture and early ripening stages."

Later investigational work by Hucker (24) has been done on the types of bacteria present in American cheddar cheese. He studied 265 cultures of bacteria obtained from 37 samples of various grades of commercial cheese. He divides these cultures into seven groups as follows: (1) spore formers, (2) gram-negative rods, (3) lactobacilli, (4) *Streptococcus lactis*, (5) cocci, (6) streptococci other than *S. lactis*, and (7) yeasts. He concludes from his study that in the higher grades of cheese, the *S. lactis* and lactobacilli were the predominating types, while in the lower grades the spore-forming and gram-negative rods were most abundant. There seemed to be little variation in the frequency of the cocci and streptococci (other than *S. lactis*) in the different qualities of cheese.

In a study of the relation of the number of bacteria in milk to the quality of cheddar cheese, Hucker (22) found that the total number of

bacteria present in milk used in the making of sixty lots of cheese had no influence upon the quality. The milk which was used varied in bacterial content from 220,000 to 41,400,000 per c.c. when it was received at the cheese factory. Under the conditions of the experiment, the milk that contained from 12 to 42 million bacteria per c.c. produced a cheese of a more constant quality than did the milk containing a smaller number of bacteria. He concludes that the specific types of bacteria present in the milk are far more important than the total number.

Judging from the above brief discussion of the bacteriology of cheddar cheese, it would seem that in order to be effective in the production of a higher quality of cheese, clarification must modify the flora of the milk, and in such a way that the groups of organisms which are responsible for the production of the desirable qualities in cheese predominate during the ripening period.

#### METHODS

The milk used in the manufacture of the experimental cheese represents the general milk supply of the Dairy Departments of the Utah Agricultural College and Iowa State College. In general, the milk was of a good quality, but off-flavored milk, and milk having a high bacterial content was occasionally used. Since the cheese was made over a period of several years in the two states, it has been possible to use milk varying as widely in quality as that employed in the various factories.

Approximately twenty gallons of milk were used for each vat, and two cheese were obtained from this amount. Immediately before using, all equipment employed was carefully steamed or rinsed with boiling water. Ten-gallon cans were used for transporting the milk from the milk vat and clarifier to the cheese vats.

In obtaining the clarified milk for the cheese, at least ten gallons of milk were run through the clarifier before the milk for the experimental work was caught.

It was the purpose throughout the trials to employ the usual commercial method of manufacture with little variation. In brief, the method generally used was as follows: One to two per cent of starter was added to the milk as soon as it had been placed in the vats. The milk in each vat was then heated simultaneously to 30°C. (86°F.) and one ounce of color and four ounces of rennet added. Cutting was done with one-quarter inch wire knives. A cooking temperature of 27.8° to 38.9°C. (82° to 102°F.) was used and the acidity at the time of draining was 0.14 to 0.16 per cent. The matting process required two or three hours, and the acidity of the whey at the time of salting was usually about 0.8 per cent or higher. Salt was added at the rate of 2.5 per cent to the curd. The curd was placed in the hoops and pressed at a temperature of 26.7° to 29.4°C. (80° to 85°F.). Usually, pressing occupied 18 to 20 hours. An average curing room temperature of 12.8° to 15.5°C. (55° to 60°F.) was maintained. The cheese were scored when they had been cured about one month and again after about three months. It was the aim to make a firm-bodied cheese having a moisture content of about 36 per cent.

When scoring, the cheese were numbered, so as to give no information to the judges on the kind of milk used for each cheese. For the purpose of making careful comparisons, the make of each day was judged separately.

Representative lots of cheese were examined for numbers and types of bacteria present. In obtaining the samples, the surface of the cheese was cut with a sterile knife, and a sterile trier was then used for securing a plug of cheese. Thin slices were cut from this and weighed on paper. About one gram of cheese was used for an analysis and this was thoroughly ground in a sterile mortar with sterile sea sand. The grinding process occupied at least twenty minutes; when completed, the material was transferred to a water blank and plated on beef infusion agar. The plates were incubated at room temperature for five to six days with the exception of those from the one month old cheese, which were incubated at 37°C. (98.6°F.) for 16 hours and then at room temperature for four days.

#### RESULTS

##### *A. Scores on Cheese from Unclarified and Clarified Milk.*

Five different series of cheese were made. Series one to four, inclusive, comprise cheese made under normal conditions, while milk containing added slime or alkali forming bacteria was used for the cheese made in series five.

##### *Summary of the Scores of Cheese in Series 1 to 4*

The summary of the results obtained in the 67 comparisons of cheese made from unclarified and clarified milk, presented in table 7, shows that clarification caused increases and decreases in the total scores of the cheese, while in a number of comparisons there was no change. An increase occurred more frequently than a decrease.

In the one month old cheese clarification resulted in an increase in the average score for flavor and aroma of all the cheese of 0.07, while in the three months old cheese it resulted in an increase of 0.41.

In the one month old cheese clarification caused no change in the average score for body and texture of all the cheese while in the three months old cheese it resulted in an increase of 0.15.

When the total scores of the cheese in all the comparisons are considered, clarification caused an increase in the average score of the one month old cheese of 0.07 and in the three months old cheese of 0.56.

##### *Summary of the Scores of Cheese in Series 5*

A. If the clarifier is to improve the quality of milk bacteriologically, it must remove undesirable bacteria from the milk and deposit them in the slime. In order to determine the effect of the addition of the slime obtained from 40 gallons of milk to 20 gallons of clarified milk on the quality of cheese obtained from unclarified milk of the same lot, six trials were made.

The total scores showed that the addition of slime caused in the one month old cheese an average decrease of 1.08, and in the three months old cheese of an average decrease of 1.25.

B. The results obtained in the study of the types of bacteria present in unclarified milk and in the clarifier slime obtained from it, which are shown in Part I, indicate that the alkali forming bacteria are the ones which are measurably removed by the clarifier.



TABLE 7. Summary of Scores of Cheese, Series 1 to 4.\*

	Flavor and aroma				Body and texture				Average total scores				Changes in average scores due to clarification						Times change in total scores due to clarification					
									For flavor and aroma, body and texture, color and finish				Flavor and aroma		Body and texture		Total score		1 month			3 months		
	Unclarified 1 month	Clarified 1 month	Unclarified 3 months	Clarified 3 months	Unclarified 1 month	Clarified 1 month	Unclarified 3 months	Clarified 3 months	Unclarified 1 month	Clarified 1 month	Unclarified 3 months	Clarified 3 months	1 month	3 months	1 month	3 months	1 month	3 months	Increase	Decrease	No change	Increase	Decrease	No change
	Av. score	Av. score	Av. score	Av. score	Av. score	Av. score	Av. score	Av. score																
Series 1 .....	37.125	37.300	36.090	36.818	29.300	29.225	29.000	29.023	91.425	91.525	90.09	90.84	+0.175	+0.727	-0.075	+0.023	+0.100	+0.75	11	8	1	14	5	3
Series 2 .....	37.950	38.300	37.900	37.950	26.900	27.300	27.300	27.700	89.850	90.600	90.200	90.650	+0.350	+0.050	+0.400	+0.400	+0.750	+0.45	7	3	0	5	5	0
Series 3 .....	39.265	39.176	39.285	39.357	27.264	27.206	27.821	27.750	91.529	91.382	92.106	92.106	-0.089	+0.072	-0.059	-0.071	-0.147	0.000	10	5	2	6	5	3
Series 4 .....	36.656	36.594	38.800	39.267	26.719	26.625	26.233	26.633	88.375	88.219	90.033	90.900	-0.062	+0.467	-0.094	+0.400	-0.156	+0.867	6	4	6	10	3	2
Total scores, series 1.....	742.5	746.0	794.0	810.0	586.0	584.5	638.0	638.5	1828.5	1830.5	1982.0	1998.5												
Number of comparisons.....	20	20	22	22	20	20	22	22	20	20	22	22												
Total scores, series 2.....	379.5	383.0	379.0	379.5	269.0	273.0	273.0	277.0	898.5	906.0	902.0	906.5												
Number of comparisons.....	10	10	10	10	10	10	10	10	10	10	10	10												
Total scores, series 3.....	667.5	666.0	550.0	551.0	463.5	462.5	389.5	388.5	1556.0	1553.5	1289.5	1289.5												
Number of comparisons.....	17	17	14	14	17	17	14	14	17	17	14	14												
Total scores, series 4.....	586.5	585.5	582.0	589.0	427.5	426.0	393.5	399.5	1414.0	1411.5	1350.0	1363.5												
Number of comparisons.....	16	16	15	15	16	16	15	15	16	16	15	15												
Av. of all comparisons.....	37.71	37.78	37.78	38.19	27.72	27.72	27.77	27.92	90.43	90.50	90.55	90.11	+0.07	+0.41	0.00	+0.15	+0.07	+0.56						

+ = Increase in score.  
- = Decrease in score.  
\*Cheese scored by Professor E. F. Goss, Dr. N. S. Golding and Mr. H. R. Lochry.





TABLE 8. *Influence of Clarification on the Numbers and Types of Bacteria Present in Cheese.*

No.		One month			Three months		
		Bacteria per gram	Inc. over undarified in percentage	Inc. in score due to clarification	Bacteria per gram	Inc. over undarified in percentage	Inc. in score due to clarification
318	U.*	1,678,000			1,100,000		
318	Cl.**	4,326,500	157.8	-0.5	2,951,000	168.3	+0.5
319	U.	3,492,600			3,268,000		
319	Cl.	1,781,000	-49.0	-5.0	6,720,000	105.6	-1.0
319	Cl. (no starter)	36,658,000	949.6		10,748,000	228.9	
320	U.	17,319,000			8,450,000		
320	Cl.	7,207,000	-58.4	-3.0	10,840,000	28.3	-0.5
320	Cl. and slime	16,380,000	-5.4		6,806,000	-19.5	
322	U.	6,920,000			4,182,000		
322	Cl.	5,701,000	-17.7	-4.0	20,770,000	396.6	-1.5
322	Cl. and slime	6,250,000	9.7		7,039,000	68.3	
323	U.	20,742,000			12,150,000		
323	Cl.	2,335,000	-88.7	+1.0	8,366,000	-31.1	+0.5
323	Cl. and slime	2,742,000	-86.8		7,096,000	-41.6	

TABLE 8. *Continued.*

No.		Percentage													
		Acid						Alkali							
		Coagulators			Non-coagulators			Inert		Alkali formers		Peptonizers			
		1 mo.	3 mo.	1 mo.	3 mo.	1 mo.	3 mo.	1 mo.	3 mo.	1 mo.	3 mo.	1 mo.	3 mo.	1 mo.	3 mo.
318	U.*	18.0	60.0	70.0	40.0	10.0									
318	Cl.**	8.0	66.0	84.0	32.0	8.0									
319	U.	10.0	70.0	70.0	30.0	20.0		2.0							
319	Cl.	22.0	78.0	72.0	22.0	4.0									
319	Cl. (no starter)	12.0	58.0	26.0	40.0	2.0									
320	U.	8.0	84.0	84.0	16.0	4.0									
320	Cl.	6.0	90.0	90.0	10.0	4.0									
320	Cl. and slime	16.0	92.0	62.0	8.0	12.0									
322	U.	14.0	56.0	86.0	44.0										
322	Cl.	36.8	90.0	59.2	8.0										
322	Cl. and slime	16.0	68.0	84.0	32.0										
323	U.	82.0	90.0	18.0	6.0										
323	Cl.	70.0	78.0	28.0	22.0										
323	Cl. and slime	42.0	82.0	56.0	16.0										

\*Unclarified.

\*\*Clarified.

A study was made of the effect on the quality of the cheese, of the addition of a pure milk culture of an alkali former to five batches of clarified milk; unclarified milk of the same lot was used for the controls.

The results obtained showed that the addition of one per cent of a milk culture of alkali forming bacteria to clarified milk caused, in general, a small decrease in the scores of the cheese when these are compared with the scores obtained from cheese made from unclarified milk of the same lot.

### B. *Bacteriological Studies*

#### 1. *Influence of Clarification of Milk on the Numbers and Types of Bacteria Present in Cheese.*

Five different lots of cheese were examined for the numbers and types of bacteria present and the findings compared with the results obtained from the scorings.

In one comparison, cheese was made from unclarified and clarified milk of the same lot; in another, from unclarified milk, clarified milk, and clarified milk containing no starter; while in three, unclarified milk, clarified milk, and clarified milk containing added slime were used. Table 8 gives the numbers of bacteria and the percentages of the various types present in the cheese when it was about one month old and also when about three months old.

In one month old cheese clarification caused an increase in the number of bacteria present in one comparison and a decrease in four, while in the three months old cheese clarification caused an increase in the number of bacteria present in four comparisons and a decrease in one.

In the one month old cheese the number of bacteria per gram in the cheese made from unclarified milk ranged from 1,678,000 to 20,742,000, and in the cheese made from clarified milk containing slime they ranged from 2,742,000 to 16,380,000. The one cheese made from clarified milk without starter contained 36,658,000.

In the three months old cheese the number of bacteria per gram of the cheese made from unclarified milk ranged from 1,100,000 to 12,150,000, in the cheese made from clarified milk 2,951,000 to 20,770,000, and in the cheese made from clarified milk containing slime from 6,806,000 to 7,096,000. These great differences in the numbers of organisms present in the cheese made from treated and untreated milk may not be as significant as they would seem. Large errors may result from the difficulty with which bacterial groups and chains present in the cheese are broken up and distributed during the grinding of the sample. Ordinarily, the number of bacteria present in cheese is high. In this study the numbers present were relatively small; probably many of the organisms had died before the cheese was examined. The period of examination would suggest this.

In the one month old cheese the numbers of bacteria in the clarified milk cheese exceeded those present in the unclarified milk cheese from —88.7 to 157.8 per cent. Adding slime to clarified milk caused increases from —86.8 to —5.4 per cent, as compared with the bacteria present in the cheese from unclarified milk, while in the cheese made from milk containing no starter there was an increase of 949.6 per cent.

In the three months old cheese the numbers of bacteria in the clarified milk cheese exceeded those present in the unclarified milk cheese from

—31.1 to 395.6 per cent, adding slime to clarified milk caused increases from —41.6 to 68.3 per cent, as compared with the bacteria present in the cheese from unclarified milk, while in the cheese made from milk containing no starter there was an increase of 228.9 per cent.

In the one month old cheese clarification caused an increase in the total score in one comparison and a decrease in four. In the comparison when there was an increase in the numbers of bacteria there was a decrease in the score. In the comparison where there was the greatest decrease in numbers of bacteria there was an increase in the score. An increase or decrease in the numbers of bacteria did not cause a similar increase or decrease in the total score.

In the three months old cheese clarification caused an increase in the total scores in two comparisons and a decrease in three. An increase or decrease in the numbers of bacteria present did not regularly cause a corresponding increase or decrease in the scores of the cheese.

The results of a study of the effect of clarification of milk and the addition of slime to clarified milk on the types of bacteria present in the cheese show that in both the one month and three months old cheese, with the exception of that made from clarified milk containing no starter, the acid forming groups constituted the majority of the organisms present; these groups were always present in the cheese made from unclarified milk, clarified milk, and clarified milk containing added slime; sometimes there was an increase and sometimes a decrease in these groups as a result of treating the milk. The alkali formers and neutral peptonizers were entirely absent. In some of the comparisons the one month old and the three months old cheese contained small numbers of inert and acid peptonizers. The one month old cheese made from clarified milk containing no starter contained a large percentage of acid peptonizers, while the three months old cheese showed a flora which was similar to that of the cheese made from the unclarified and clarified milk of the same lot.

In summarizing these results, it would appear that clarification of milk and the addition of slime to clarified milk had no specific influence on the numbers and types of bacteria present in the cheese, and that an increase or decrease in the numbers of bacteria present in the cheese obtained from the treated milk as compared with the numbers present in the cheese from the untreated milk of the same lot did not result in a corresponding constant increase or decrease in the total scores of the cheese.

## 2. *Relation Between the Numbers of Bacteria Present in Unclarified and Clarified Milk and the Quality of Cheese Obtained.*

In order to determine the relation between the numbers of bacteria present in the unclarified and clarified milk used and the quality of the cheese made, 28 comparisons were made. The cheese were made during the period May to July, 1927, inclusive.

Table 9 shows the plate counts of the milk used, the percentage change in numbers due to clarification, and the increase in the total scores of the cheese caused by clarification. The counts of the unclarified milk ranged from 20,900 to 15,010,000 per c.c. Clarification caused an increase in the bacterial count in three trials and a decrease in 25. The increases in the counts varied from 5.0 to 23.8 per cent and averaged 11.8 per cent, while



TABLE 9. *Relation Between Numbers of Bacteria Present in Unclarified and Clarified Milk and the Quality of Cheese Obtained.*

Number	Unclarified	Clarified	Percentage		Increase in total score due to clarification	
			Increase	Decrease	1 month	3 months
512	20,900	19,800		5.3	1.0	-0.5
513	530,000	485,000		8.5	0.5	0.0
514	350,000	250,000		28.6	-1.0	0.5
516	710,000	530,000		25.4	0.5	0.0
521	11,470,000	9,480,000		17.4	2.0	0.5
523	11,400,000	9,160,000		19.7	1.5	-0.5
528	1,160,000	880,000		24.1	0.5	0.0
606	200,000	210,000	5.0		1.0	1.0
607	63,000	78,000	23.8		2.0	—
608	1,080,000	590,000		45.4	0.0	—
609	1,220,000	745,000		38.9	0.0	1.0
611	1,810,000	1,360,000		24.3	1.0	—
613	490,000	360,000		26.5	0.0	4.0
614	260,000	190,000		26.9	0.0	1.0
615	137,000	119,000		13.1	0.5	1.0
616	114,000	107,000		6.1	0.0	-1.0
617	3,290,000	2,630,000		20.1	0.0	0.0
620	2,480,000	2,340,000		5.6	0.0	-0.5
621	15,010,000	7,620,000		49.2	-3.0	0.5
622	560,000	400,000		28.6	-2.5	-2.0
623	8,180,000	5,340,000		34.7	-1.5	0.0
624	4,200,000	2,000,000		52.4	0.0	0.5
627	1,490,000	820,000		45.0	2.0	4.0
628	2,210,000	1,635,000		26.0	0.5	3.0
629	1,960,000	2,090,000	6.6		1.0	1.0
630	1,170,000	980,000		16.2	0.5	0.5
701	875,000	410,000		53.1	0.5	1.0
705	11,800,000	10,600,000		10.2	—	—

the decreases varied from 5.3 to 53.1 per cent and averaged 26.1 per cent. Considering the 28 comparisons, there was an average decrease of 22.0 per cent as a result of clarification. The results are comparable with those obtained in Part I, where clarification caused increases and decreases in the counts, a decrease being caused more often when the number of bacteria per c.c. exceeded 100,000.

With the smallest increase in bacterial count, as a result of clarification, there was an increase in the total score of the one month old cheese of 1.0, and an increase in the total score of the three months old cheese of 1.0. With the largest increase in the count, there was an increase in the total score of the one month old cheese of 2.0. The three months old cheese in this comparison was not scored.

With the smallest decrease in the bacterial count, as a result of clarification, there was an increase in the total score of the one month old cheese of 1.0, and a decrease in the total score of the three months old cheese of 0.5. With the largest decrease in the count, there was an increase in the total score of the one month old cheese of 0.5 and an increase in the total score of the three months old cheese of 1.0.

When all the comparisons are considered, there was an average increase in the total score of the one month old cheese of 0.23 and an increase in the total score of the three months old cheese of 0.60.

Comparisons Nos. 613, 627 and 628 show the largest increase in the total score of the cheese and clarification caused a decrease in numbers of bacteria in the milk used of 26.5, 45.0, and 26.0 per cent, respectively. Comparisons Nos. 621 and 622 show the largest decreases in the total score of the cheese and in these clarification caused a decrease in the numbers of bacteria in the milk used of 49.2 and 28.6 per cent, respectively.

These results show that increases or decreases in the numbers of bacteria present in the milk as a result of clarification did not have definite effects on the score of the cheese.

## SUMMARY

### PART I

1. Forty-three comparisons of the effect of clarification on the numbers of bacteria in milk showed that clarification caused an increase in the counts in 20 trials and a decrease in 23 trials. The increases in the counts varied from 3.1 to 143.7 per cent and averaged 54.6 per cent, while the decreases varied from 0.4 to 59.1 per cent and averaged 24.9 per cent. Considering the 43 comparisons, clarification caused an average increase of 12.1 per cent. With the milk studied, clarification more often caused a decrease in the count in the milk containing over 100,000 bacteria per c.c. than when the number was smaller.

2. Nineteen comparisons of the influence of clarification on the number of bacteria in milk, using a normal and a reduced rate of inflow, showed that with a normal rate of inflow clarification caused an increase in the counts in six trials and a decrease in 13, but when the rate of inflow was reduced, clarification always caused a decrease. With the normal rate of inflow the increases varied from 5.9 to 64.8 per cent and averaged 35.1 per cent, while the decreases varied from 3.4 to 60.2 per cent and averaged 20.8 per cent. Considering the 19 comparisons, there was an average decrease of 3.1 per cent. When the rate of inflow was reduced there was a minimum decrease of 25.9 per cent, a maximum of 80.5 and an average of 55.3 per cent.

3. Twenty-one comparisons of the influence of clarification on the types of bacteria present in milk showed that clarification had an irregular effect on the bacterial flora. Sometimes it caused an increase and sometimes a decrease in each of the groups of organisms present in the milk. The increases in the acid coagulators varied from 0.3 to 34.3 per cent and the decreases from 0.2 to 23.7 per cent, with an average increase, when all the comparisons are considered, of 3.4 per cent. The acid non-coagulators showed increases varying from 0.8 to 24.0 per cent, decreases from 0.8 to 16.0 per cent, and an average increase in all comparisons of 3.9 per cent. The alkali formers were increased from 0.1 to 16.0 per cent and decreased from 1.2 to 22.0 per cent, with an average decrease for all comparisons of 1.8 per cent. The other groups showed similar increases and decreases.

4. Thirty-three comparisons of the types of bacteria present in unclarified milk and in the slime obtained from it showed that the only groups which indicated a fairly consistent change as a result of clarification were the acid coagulators and the alkali formers. The average changes were an 8.7 per cent decrease in the percentage of acid formers and a 16.8 per cent

increase in the percentage of alkali formers in the slime as compared with the milk.

5. The results of a series of 18 comparisons on the effect of clarification with a normal and a reduced rate of inflow on the types of bacteria in milk showed that clarification with both methods caused little change in the types of bacteria present. The variations were not consistent or large enough to permit of any conclusions other than that the clarifier had no pronounced selective action on the types of bacteria present in the milk under either method of clarification.

6. In nine comparisons clarification under a normal and under a reduced rate of inflow caused an increase in the time required to reduce methylene blue, when unclarified milk was used as the basis for comparison. The average increases in the reduction time when all the comparisons are considered were 21 minutes with a normal rate of inflow and 47 minutes with clarification under a reduced rate of inflow. The results agreed, in general, with those obtained by the plate method, but the latter method was more refined in showing the changes in the numbers of bacteria of milk resulting from clarification. Observations of the fermentation test indicated that clarification with both rates of inflow had little effect on the fermentation occurring.

7. When raw milk was subjected to centrifugal force in centrifuge tubes for various periods of time, it was found in four comparisons that five minutes centrifuging removed an average of 12.6 per cent of the bacteria present, while 20 minutes exposure to the centrifugal force removed an average of 16.7 per cent in the sediment. The centrifuging of samples of milk of the same lot for five, ten and thirty minutes, respectively, in four comparisons, and for five and twenty minutes in four other comparisons caused only slight changes in the bacterial flora; whenever a certain type of organism was present in the milk it was also found in the sediment. Even an exposure of the milk to centrifugal force for 30 minutes did not cause a complete elimination of any of the types of bacteria present in the milk. By subjecting raw milk to centrifugal force in tubes, it was possible to remove a large percentage of alkali forming organisms or yeasts which had been inoculated into it from agar slopes.

8. The specific gravity of *Torula cremoris*, when grown on agar, was found in four determinations to vary from 1.037 to 1.102, with an average of 1.079. Various cultures of alkali forming bacteria grown on agar were found in 13 determinations to have specific gravities ranging from 1.029 to 1.123 and averaging 1.0497, while when bouillon was the medium, the specific gravities in two determinations were 1.025 and 1.032, with an average of 1.0285.

9. A morphological, cultural and biochemical study of 31 cultures of alkali forming bacteria showed that the differences exhibited were too small to permit a division into varieties of the species to which they belong.

## PART II

1. The study of the influence of clarification on the quality of cheese made from 67 different lots of milk showed that in the one month old cheese clarification resulted in an increase of 0.07 in the average score for flavor and aroma of all the cheese, while it resulted in an increase of 0.41 in the

three months old cheese. In the one month old cheese clarification caused no change in the average score for body and texture of all the cheese, while in the three months old cheese it resulted in an increase of 0.15. When the total scores of the cheese in all the comparisons are considered, clarification caused an increase of 0.07 in the average score of the one month old cheese and an increase of 0.56 in the three months old cheese.

2. In six comparisons the addition of clarifier slime to clarified milk caused, on an average, a decrease in the quality of the cheese. In the one month old cheese it caused an average decrease of 1.08 in the total score, and in the three months old cheese an average decrease of 1.25.

In five comparisons the addition of a pure culture of alkali forming bacteria to clarified milk caused an average decrease of 1.20 in the total scores of the one month old cheese, and an average decrease of 0.40 in the three months old cheese.

3. Clarification of milk and the addition of slime to clarified milk did not have any specific influence on the numbers and types of bacteria present in the resultant cheese. An increase or decrease in the numbers of bacteria in the cheese obtained from the treated milk as compared with the numbers present in the cheese from the untreated milk of the same lot did not result in a corresponding increase or decrease in the total scores of the cheese.

4. Clarification caused increases and decreases in the numbers of bacteria present in milk, but corresponding increases or decreases in the scores of the cheese did not regularly follow these.

#### BIBLIOGRAPHY

- (1) AYERS, S. H., P. RUPP AND W. T. JOHNSON, JR.  
1919. A study of the alkali-forming bacteria found in milk. U. S. Dept. Agric. Bull. 782, 38 pp.
- (2) BABCOCK, S. M.  
1894. Cleaning milk with a centrifugal separator. Wis. Agric. Exp. Sta. 11th Ann. Rept., 146-149.
- (3) BAHLMAN, CLARENCE.  
1916. Milk clarifiers. Am. Jour. Pub. Health, 6:854-57.
- (4) BERGEY, D. H.  
1925. Bergey's manual of determinative bacteriology. (Second Edition.) Williams and Wilkins, Baltimore. 462 pp.
- (5) COMBS, W. B., W. H. MARTIN AND N. A. HUGGLAR.  
1924. Clarification of milk for cheesemaking. Jour. of Dairy Science, 7:524-529.
- (6) DAHLBERG, A. C., AND J. C. MARQUARDT.  
1924. Filtration and clarification of milk. New York State Agric. Exp. Sta. Tech. Bull. 104, 27 pp.
- (7) DEAN, H. H.  
1903. Report of the professor of dairy husbandry. Ontario Agric. College and Exp. Farm, 28th Ann. Rpt., 61-63.
- (8) DOANE, C. F.  
1903. Economical methods for improving the keeping qualities of milk. Maryland Agric. Exp. Sta. Bull. 88:117-164.



- (9) DUCLAUX, E.  
1878. Fabrication, maturation et maladies due fromage du Cantal. *Ann. Agron.*, 4:5-18.
- (10) DUNBAR, DR., AND I. KISTER.  
1899. Versuche zur Reinigung von Milch. *Milch-Zeitung*, 50:787.
- (11) ECKLES, C. H. AND S. E. BARNES.  
1901. Purification of milk by centrifugal separation. *Iowa Agric. Exp. Sta. Bull.* 59:55-59.
- (12) ERNST, WM.  
1914. Text book of milk hygiene. (Translated and revised by Mohler and Eichhorn), p. 150.
- (13) EVANS, A. C., E. G. HASTINGS AND E. B. HART.  
1914. Bacteria concerned in the production of the characteristic flavor in cheese of the cheddar type. *Jour. Agric. Res.*, 2:167-192.
- (14) FISK, WALTER C., AND WALTER V. PRICE.  
1923. The clarification of milk for cheese making. New York (Cornell) *Agric. Exp. Sta. Bull.* 418, 22 pp.
- (15) FLEISCHMAN, W.  
1896. The book of the dairy. (Translated by C. M. Aikman and R. P. Wright), 97-98.
- (16) GROTENFELT, G.  
1894. The principles of modern dairy practice. (Translated by F. W. Woll), 183-187.
- (17) HAMMER, B. W.  
1916. Studies on the clarification of milk. *Iowa Agric. Exp. Sta. Resch. Bull.* 28, 19-32.
- (18) HAMMER, B. W., AND A. J. HAUSER.  
1918. Studies on the clarification of milk—II. *Iowa Agric. Exp. Sta. Resch. Bull.* 47, 79-97.
- (19) HAMMER, B. W., AND M. P. BAKER.  
1926. Classification of the *Streptococcus lactis* group. *Iowa Agric. Exp. Sta. Resch. Bull.* 99:283-300.
- (20) HARDELL, R. E.  
1927. Ohio Swiss cheese progress. *Chicago Dairy Produce*, 33, No. 40: 22-23.
- (21) HINKELMAN, A. J.  
1916. Micro-organic weight. *Ill. Med. Jour.* 29:202..
- (22) HUCKER, G. J.  
1921. Relation of the number of bacteria in milk to the quality and yield of cheese. New York (Geneva) *Agric. Exp. Sta. Bull.* 486, 19 pp.
- (23) —————  
1921. The types of bacteria found in commercial cheddar cheese. New York *Agric. Exp. Sta. Tech. Bull.* 90, 38 pp.
- (24) —————  
1922. Review of the bacteriological aspects of cheese ripening. New York *Agric. Exp. Sta. Tech. Bull.* 89, 36 pp.
- (25) JUDKINS, H. F., AND P. A. DOWNS.  
1918. Studies in processing milk. Conn. (Storrs) *Agric. Exp. Sta. Bull.* 99, pp. 449-470.

- (26) MARPMANN, G.  
1903. Ueber die Reinigung der Milch von Tuberkelbazillen durch Zentrifugierung. *Milch-Zeitung*, 41:642.
- (27) MARSHALL, C. E., E. G. HOOD, ET AL.  
1918. Clarification of milk. *Mass. Agric. Exp. Sta. Bull.* 187:155-242.
- (28) MARSHALL, C. E., E. G. HOOD, ET AL.  
1920. Clarification of milk. *Jour. Dairy Science*, 3, No. 4:245-259.
- (29) MATHESON, J. K.  
1924. The effect of centrifuging milk for the manufacture of Swiss cheese. *Proceedings of the World's Dairy Congress*, 1:292-296.
- (30) MCCLINTOCK, J. A.  
1916. An investigation of clarification of milk. *The Milk Trade Journal*, 4, No. 6:8.
- (31) —————  
1916. Further investigations upon the clarification of milk. *Milk Trade Journal*, 4, No. 7:64.
- (32) MCINERNEY, T. J.  
1917. Clarification of milk. *New York (Cornell) Agric. Exp. Sta. Bull.* 389:487-504.
- (33) MOORE, V. A.  
1895. Inefficiency of milk separators in removing bacteria. *Yearbook. U. S. Dept. Agric.* 1895:431-444.
- (34) OLSON, T. M.  
1926. Milk clarifiers and milk filters. *So. Dakota Agric. Exp. Sta. Ann. Rpt.*, 16-17.
- (35) PARKER, H. N.  
1917. City milk supply. *McGraw-Hill Book Co., New York*, 256-258.
- (36) REPORT OF DIRECTOR.  
1926. Clarification of milk. *Idaho Agric. Exp. Sta. Bull.* 142:16.
- (37) SEVERIN, S. A.  
1905. Vermindert die Centrifugierung die Bakterienzahl in der Milch? *Centralbl. f. Bakt. II Abt.*, 14:605-15.
- (38) SHERMAN, J. M.  
1916. Studies on the production of sanitary milk. *Penn. Agric. Exp. Sta. Ann. Rpt.* 1915:299-305.
- (39) —————  
1917. Some bacteriological tests of the milk clarifier. *Jour. Dairy Science*, 1:272-278.
- (40) STANDARD METHODS OF MILK ANALYSIS (Fourth Edition)  
1923. *Am. Pub. Health Assn.*
- (41) TRAUM, J., AND G. H. HART.  
1916. The value of efficient commercial pasteurization in safeguarding milk naturally infected with tubercle bacilli. *Jour. Amer. Vet. Med. Assn.*, 49, (n. s.) 2:678-698.
- (42) WARDLAW, H. S. H.  
1914. On the nature of deposit obtained from milk by spinning in a centrifuge. *Jour. and Proc. Roy. Soc. of New So. Wales*, 48:152-171.



# EXPERIMENTS ON THE PHYSIOLOGICAL RELATIONSHIPS BETWEEN THE STOMACH INFUSORIA OF RUMINANTS AND THEIR HOSTS, WITH A BIBLIOGRAPHY

ELERY R. BECKER, J. A. SCHULZ AND M. A. EMMERSON\*

*From the Department of Zoology; Chemistry Section, Agricultural Experiment Station;  
and Department of Veterinary Anatomy, Iowa State College.*

Accepted for publication November 5, 1929.

The general problem of host-parasite relationships has captivated present day workers in protozoology. One of the most outstanding pieces of work in this field was that by Cleveland (1924), which has apparently demonstrated that certain termites are dependent upon the protozoan fauna of their digestive tract for the digestion of the cellulose in their woody diet. These protozoa are thus true symbionts. The zoologist is naturally curious to know whether or not other mutually beneficial associations between entozoic protozoa and their metazoan hosts occur in nature. After Cleveland's paper was published, the question was frequently asked, "Do the infusoria in the paunch of a ruminant assist their host in a similar manner?" We find that the same inquiring frame of mind held Gruby and Delafond, who in 1843 discovered the protozoan fauna of the rumen and reticulum of domestic ruminants (also that of the caecum and colon of the horse) during their investigations on digestion. Their opinions will be presented later, but the point we wish to emphasize here is that they, and most of the investigators since them who have studied this stomach fauna characteristic of ruminants, have ventured either to express an opinion concerning the physiological rôle of these organisms in their host or to mention the statements of others regarding them.

We find after a thorough review of the literature that various views have been held regarding the value of the rumen infusoria to their host. These views may be stated briefly as follows: (1) The rumen infusoria convert substances in plant food into the more easily digestible animal substance of their own bodies, which in turn are sacrificed to the action of the digestive juices of their host; (2) they are harmful parasites; (3) they are harmless commensals; (4) they check the increase of Schizomycetes which would eventually become harmful to their host; (5) they assist in the digestion of cellulose; (6) they are valuable to their host in mechanical ways; namely, the thorough mixing and trituration of the rumen contents. Each topic will be discussed separately in the following paragraphs.

---

\*The funds which made this work possible were supplied by grants from both the American Association for the Advancement of Science and the Bache Fund of the National Academy of Sciences. We are likewise indebted to many individuals on the Iowa State College faculty for various kinds of information and other valuable assistance. Especially to Professors E. M. Mervine, H. D. Bergman, H. H. Dukes, R. E. Buchanan and C. J. Drake do we wish to express our appreciation and thanks.



### 1. *Conversion of Plant Substances Into Animal Substances.*

Gruby and Delafond (1843) felt that the presence of such vast numbers of microörganisms in the stomach of an animal during the digestion of food indicated that they held a certain importance in the digestive processes of their host. This thought would naturally occur to them, for they were working on the general problem of digestion in domestic animals; and the important discovery of the existence of these most complex protozoa was merely incidental to their principal objective. They observed that the protozoa occupied only the rumen and reticulum of the stomach, and that those which were carried with the food mass into the true stomach were digested there. They reasoned in an empirical way: the ruminant is a herbivorous animal; these protozoa grow upon plant nutrients, which are converted into animal protoplasm; this in turn is digested by the ruminant, making it in a certain sense carnivorous.

This general theory takes more definite and modern form in the recent work of Schwarz (1925). To him the protozoa play the rôle of converting amides and protein derivatives, difficult to digest, into easily digestible animal protoplasm. He writes, "Es erscheint daher nicht ausgeschlossen, dass diese Mikroorganismen, wie bereits früher von den oben genannten Autoren für möglich gehalten wurde, eine bedeutsame Nahrungsquelle für die Wiederkäuer darstellen, wodurch zum Beispiel die in den Pflanzen vorkommenden Amide und die tiefen, durch Bakterientätigkeit erhaltenen Eiweiss Spaltprodukte in einer wieder verwertbaren Form als Infusorienprotoplasma gestapelt werden." He finds upon chemical analysis that 20 per cent of the nitrogen in the rumen contents is stored up in the infusoria and about 11.7 per cent in the bacteria. After certain calculations upon the protein requirements of ruminants, he concludes that the microörganisms in the anterior portion of the stomach of ruminants have a relatively large, if not predominant, importance as sources of nourishment. In a later paper Schwarz and Bienert (1926) expressed a similar view of the situation regarding the infusoria in the caecum of the horse.

Biedermann (1911) says that the infusoria are of no important use as protein converters. Scheunert (1924) believes that they are not present in sufficient amounts to be of any great importance. Mangold and Schmitt-Krahmer (1927), employing the method of analyzing rumen contents of sheep comparatively infusoria-free and normal sheep, find nothing like the 20 per cent increase in N content of the latter over the former, as would be expected from the results of Schwarz. The most recent work on this subject is by Ferber (1928), whose results lead him to believe that the infusoria may have some rôle in converting the proteins in the plant food into more easily digestible infusorian protoplasm, but that they cannot convert amides into proteins, as reported by Schwarz. Later, Ferber and Winogradowa-Fedorowa (1929) state: "Diese Tatsache scheint uns, neben den oben erwähnten, dafür zu sprechen und zu bestätigen, dass den Panseninfusorien eine wichtige physiologische Bedeutung und eine notwendige Rolle für die Ernährung ihrer Wirtstiere zukommt." Buisson (1923) suggests that they supply an animal element in the ration of herbivorous animals.

Later we present some of our results bearing upon the foregoing problem, although it is by no means settled.

## 2. *Injurious Parasites.*

So far as we can learn, the view that the stomach infusoria are injurious to their host has been held only by Zürn (1887). He thought that they could lead to the development of pathological conditions in the alimentary track (*Magen- und Darmkatarrh*). Subsequent investigators have learned that his opinion was incorrect, for the occurrence of these protozoa in almost every normal healthy ruminant seems to imply a well-being of the host, rather than a diseased condition (Cf. Trier, 1926, p. 307). (This is probably because sick animals eat little or no food, and under these conditions the numbers of infusoria decrease very rapidly.)

## 3. *Harmless Commensals.*

Bundle (1895), Biedermann (1911), Scheunert (1924), Scheunert and Schielblieh (1927) take the position that these protozoa are harmless commensals. If they are of any value to their host, it is in mechanical ways. Likewise, Weiss (1869) presumably held the commensal idea, for he opposed Gruby and Delafond's idea that they were of value in the digestive processes.

## 4. *Reduction of Schizomycetes.*

The idea that the rumen infusoria might be useful as scavengers was original with List (1885). The ingestion by the infusoria of bacteria and moulds on a large scale would serve to prevent their inordinate multiplication, which would eventually become dangerous to the animal organism.

Why does not the rumen content of an ox, with its twenty or thirty gallons of water, saliva, hay and grain, at a favorable temperature for bacterial growth, and teeming with bacteria, become a putrefying mass? This, as yet, has not been explained fully.

## 5. *Digestion of Cellulose.*

It has long been known that ruminants digest a considerable amount of the cellulose in their diet of hay, grasses and grains. Schuberg (1888) observed that *Entodinium* and *Diplodinium* ingest plant particles, some of them so large as to distort the outline of the cell. He concludes, "dass die Ciliaten speziell für die Celluloseverdauung von Bedeutung sein könnten." Eberlein (1895) noted the ingestion of plant fibre by certain of the rumen infusoria, and its subsequent disintegration within them. He concluded, as others have done since, that these infusoria could digest cellulose. In large numbers they transform a part of the cellulose into a digestible substance. The protozoa are ultimately digested in the stomach and intestine, raising the metabolism of their host. Previously, Certes (1889) had made the more sweeping statement that the protozoa condition the fermentative processes in the rumen. This writer also observed that glycogen from carbohydrate digestion was stored up in the protoplasm of certain of these unicellular organisms.

Liebetanz (1910) is in almost complete accord with Eberlein. Braune (1913) expresses his agreement with the views of Eberlein and Schuberg regarding cellulose digestion.

Reichenow (1927, p. 377), in the new edition of Doflein, immediately after the discussion of the physiological importance of termite protozoa in

the digestion of cellulose, states: "Eine ähnliche ernährungsphysiologische Rolle spielen offenbar die Ciliaten aus der Familie der Ophryoseoleciden, die in ungeheuren Mengen in den Verdauungsorganen von Säugetieren mit besonders cellulosereicher Kost vorkommen." He also discusses the importance of the bacteria in cellulose digestion in ruminants.

Cleveland (1924, p. 220), in his classic work proving the importance of the intestinal protozoa of termites in cellulose digestion within their host, wrote: "Intestinal bacteria and fungi quite often aid their vertebrate and invertebrate hosts in the digestion of cellulose and, since it has been shown in the present investigation that intestinal protozoa can digest cellulose, it is now possible that the infusoria, such as *Diplodinium*, *Entodinium*, *Buetschlia*, *Isotricha*, *Dasytricha*, and *Ophryoscolex*, harbored by ruminants, notably the ox, goat, sheep, camel and reindeer, may aid their hosts in the digestion of cellulose and hemicellulose."

A number of other scientific authorities could be quoted as evidence that the infusoria of ruminants have been seriously considered as valuable aids to their hosts in the digestion of cellulose, although the activity of bacteria in the same direction is not denied. One of our late prominent authors (Slosson, 1928), who was noted for his ability to present scientific work to a popular audience, wrote: "Even the goat that pastures on billboards and the ox that eats straw have to depend upon minute forms of *animal* (italics ours) or plant life that inhabit the digestive tract."

Trier (1926, p. 308) makes the suggestion that there may be bacteria which live in symbiosis with the protozoa and assist the unicellular organism in the digestion of cellulose. He writes: "So wäre es denkbar, dass hier eine direkte Symbiose vorliegt und zellulosebakterien und Infusorien voneinander abhängig sein. Wie weit sich die beiden Vertreter der Kleinlebewelt von pflanzlichen Teilen nähren und ob dabei eine gegenseitige Unterstützung stattfindet, bedarf noch der Klärung. Wenn hier eine zwingende Symbiose nachgewiesen würde, wäre der Beweis erbracht, dass die Infusorien die bakteriellen Gärungsvorgänge unterstützen und so dem Wiederkäuer indirekt von Nutzen sind."

We find, however, that there is no general agreement that the infusoria do assist in cellulose digestion. Bundle (1895) states if they are of any value in this way, it is not appreciable. Biedermann (1911), Scheunert (1924), and Scheunert and Schieblich (1927) are inclined to the view that since a usefulness to their host has not yet been proved, they must still regard these protozoa as commensals, except for some minor mechanical services which they might render their host. It seems to us highly desirable that it be definitely determined whether or not intracellular digestion of cellulose actually takes place in the rumen infusoria. Some of the previously mentioned early workers, as well as the more recent ones, notably Schulze (1924, 1927) and Trier (1928), claim to have seen plant fibers of a cellulose nature reduced to a fine detritus in the endoplasm. The detritus was later expelled from the anus. Dogiel and Fedorowa (1925, pp. 106-107) deny that any disintegration of cellulose elements takes place inside the infusoria. They assert that such particles are expelled from the cell in the same condition as when they were ingested. When this phase of the problem is restudied, appropriate tests should be used to determine definitely that the elements reduced are true cellulose. It is possible that certain plant elements undergo disintegration, while others do not.



### 6. *Mechanical Services.*

It appears to Bundle (1895), Scheunert (1924), and Scheunert and Schieblieh (1927) that the only service which these microorganisms render their host is purely mechanical assistance in soaking (*Quellung*), maceration (*Mazeration*) and thorough mixing (*Durchmischung*) of rumen contents. They do not venture to suggest what would happen if the protozoa were absent.

We have thus summarized the views that have been held by workers in the fields of protozoology, physiology, and veterinary medicine regarding the rôle played by rumen infusoria. Not so much attention has been paid to flagellates and amoebae, for they ingest comparatively small, if any, amounts of cellulose; and most of them are representatives of types widely distributed in nature, rather than members of the peculiarly characteristic rumen infusoria. (Scheunert, 1924, states that members of the genera *Buetschlia*, *Isotricha*, and *Diplodinium* may be found also in the esophageal portion of the stomach of the hamster.) At any rate, it is but natural to ascribe some importance to microorganisms which live, move, and multiply in enormous numbers in a portion of the digestive tract where the important processes of digestion of cellulose and other carbohydrates, and possibly also pterin and vitamin B (*Vide* Bechdel *et alii*, 1928) synthesis, take place. If the infusoria bear any relationship to these processes, economic aspects of the problems are quite evident. It has been our purpose to conduct an investigation of the general problem which would throw as much light as possible upon each of the six hypotheses previously advanced by other workers.

### RATIONALE AND TECHNIQUE OF THE EXPERIMENTS

The experiments were planned so that we might gain the information sought by comparing the results obtained in animals, for a period during which they carried the normal infection of infusoria, with a period during which they were free of the infusoria, both periods being as nearly identical as possible in respect to other conditions. The infusoria-free trials were made first, however, because we feared that otherwise the effects of the rather rigorous process of defaunation might introduce certain conditions which would complicate the results. In the digestion trials, the digestibility coefficients of protein, ether extract, nitrogen-free extract, crude fiber, hemicellulose, pentosans, alpha cellulose, and total dry matter were determined for both the infusoria-free period, and the one during which the animals carried the infection, hereafter designated the infected period. The partition of nitrogen between feces and urine, as well as the amounts of nitrogen stored daily, were also calculated for both periods.

The goat was chosen in preference to other ruminants because it is said to lend itself to laboratory conditions better than either the ox or sheep. It was decided to use two young and two adult goats for our digestion trials. We were unable to learn the exact ages of the animals, but Goats 1 and 2 were considerably under a year of age, while Goats 3 and 4 were from three to five years. These animals remained healthy throughout the experiment, and at no time was there a refusal of food, making supplementary analysis necessary. They were kept in a large steam-heated room with a cement floor. By properly disposing of excrement and flushing the



floor with a hose the room was kept sufficiently sanitary so that it gave little offense to people working in the same building.

Procuring animals infected with infusoria presented no difficulty, because, as is well known, virtually every ruminant is infected with at least some species of the genera *Entodinium*, *Diplodinium*, *Ophryoscolex*, *Isotricha*, *Dasytricha*, or *Buetschlia*. The problem of accomplishing a complete removal of infusoria from the rumen and reticulum was solved by two techniques, which have been discussed elsewhere (Becker, 1929). Two of our animals were defaunated by a method similar to that employed by Liebetanz (1910). His technic may be described briefly as follows: first, the animal was starved three days; at the end of this period a trocar was inserted through the body wall into the rumen; then the final defaunation was accomplished by introducing into the rumen a certain amount of dilute acetic acid, both the amount and the dilution having been previously experimentally determined. We employed his technique with modifications as follows: first, the goat was starved for three days, except that water was given *ad libitum*; then a trocar was inserted into the paunch through the body wall just back of the last rib, as shown in figure 1; immediately 300 c.c. of five per cent acetic acid for half grown goats, or 500 to 600 c.c. for adults, was poured into the rumen through the cannula of the trocar. The following day a mixture consisting of six eggs and a quart of milk was injected into the rumen. The putrefaction of the eggs presumably assisted the acid in killing the protozoa. As soon as the animal would eat, it was given grain and hay. By this method we succeeded in effecting a complete removal of the infusoria in the two half-grown goats; but, like Liebetanz, we were unsuccessful in the case of adult goats, even though the acetic acid dosage was repeated on successive days. The acid sometimes caused reflex vomiting, and when this happened the goat usually died soon afterwards, perhaps from a mechanical pneumonia caused by getting the acid into the lungs. We lost several goats in this way. This method of defaunation is too rigorous and dangerous for ordinary work, although occasionally it may be used successfully. The locations of the various compartments of the goat stomach are shown in figure 1. For information concerning the physiology of the ruminant stomach we consulted Schalk and Amadon (1928).

We tried varying amounts of a ten per cent solution of thymol in chloroform introduced into the rumen through the cannula, but we were not successful in killing all the protozoa in one adult goat. Finally, 6 c.c. of this solution was administered. A few minutes later the animal was found dead, but some of the infusoria were still alive.

The use of  $\text{CuSO}_4$  as an infusoriacidal agent was found to be not only more effective than the other substances tried, but it also produced less deleterious effects upon the goat. The method is as follows: first, the animal was not permitted to eat for 72 hours, but water was constantly kept before it; then 50 c.c. of two per cent  $\text{CuSO}_4$  (freshly made) in a pint of distilled water was introduced into the rumen through a rubber tube down the esophagus; another day passed during which the goat was still unfed; then followed another administration of  $\text{CuSO}_4$  solution, like the first. Several hours later feed was offered. Microscopic examination of rumen samples was made daily for at least two or three weeks to check up on the

effectiveness of the treatment. Once removed, the infusoria did not return if feed thoroughly dried and water taken directly from the tap were given (Cf. Becker and Hsiung, 1929).

After completion of the defaunation process, there followed a period of reconditioning the animal of about three weeks duration. During this period it received the same kind of hay and grain mixture that was to be used during the digestion trials. By carefully weighing the food materials a close estimate was made of how much the animal would consume each day and still maintain a keen appetite.

Next, the goat was put into a metabolism crate (described below) for seven days, and given daily the determined amount of alfalfa hay and grain

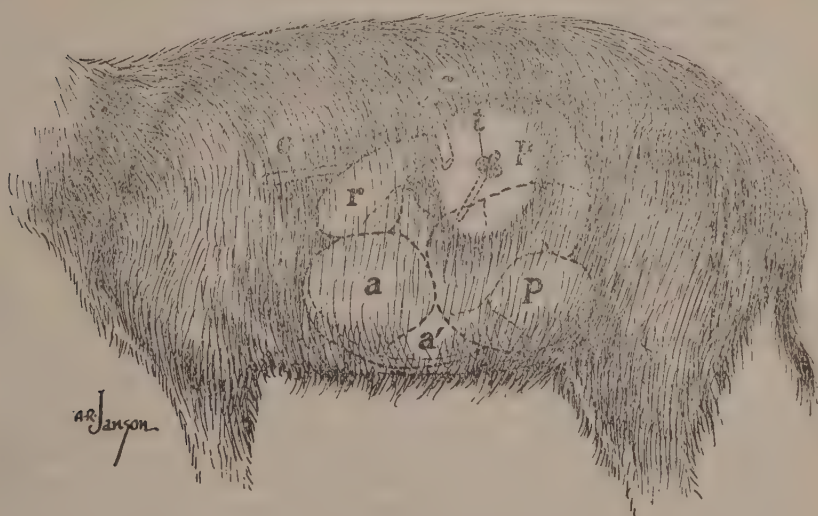


Figure 1. Goat stomach with cannula of trocar (*t*) in the upper compartment of the rumen (*P*). *c*, last rib; *O*, esophagus; *r*, reticulum; *a*, abomasum or true stomach.

mixture. Water was given *ad libitum*. The animal was fed half its daily ration of hay at 9 a. m. and the other half at 4 p. m. The grain was fed at noon. This preliminary week of feeding was to thoroughly adjust the goat to its diet. It should be mentioned at this point that the alfalfa hay had been previously chopped into inch lengths by means of an alfalfa chopper, thoroughly mixed and weighed into paper sacks in 1000 gram amounts. Some evaporation of moisture took place, of course, but each sack of hay was weighed just before feeding and evaporation was allowed for. If, for example, a goat was to get 400 grams of chopped hay per feeding, and it was discovered that there had been a moisture loss of 100 grams from the sack, the animal was given four-tenths of the remaining 900 grams of hay. Thus the amounts of the solid content of the hay fed were kept comparable throughout each experiment, regardless of moisture changes.

The grain mixture was likewise thoroughly mixed in a mechanical mixer and weighed out into paper sacks before the experiment was begun.

The grain mixture received by Goat 1 consisted of 50 parts ground oats, 50 parts cracked corn, 20 parts wheat bran, and 20 parts of a popular brand of calf meal. Salt was given *ad libitum*. Goats 2, 3 and 4 received a mixture of 100 parts ground oats, 100 parts cracked corn, 50 parts wheat bran, 10 parts linseed oil meal, 3 parts bone meal, 3 parts limestone, and  $1\frac{1}{2}$  parts common salt.

After a week of adjusting itself to its daily rations, the animal was ready for its digestion trials. These were 14 days each for the infusoria-free and the infected periods in the case of two goats, and 21 days each in the case of the other two. The feces and urine were carefully collected daily. The fecal pellets were dried over a hot water bath and stored in large friction-top cans. At the close of the period they were spread out in a thin layer for several days, weighed in an air dry condition, ground to pass through an 80 mesh sieve, and aliquoted for analysis. The urine was kept in large five-gallon bottles, into which several c.c. of a 10 per cent thymol in chloroform mixture were added daily for a preservative. Precipitation of phosphates was prevented by daily additions of small amounts of acetic acid. If the collection for a period began on a certain day at 11 o'clock, it was finished on the last day of the period at the same hour. The composite samples of urine and wash water were also filtered, weighed, and aliquoted for analysis at the end of each period.

As was mentioned above, the infusoria-free period was in all cases the first one. Frequent examinations were made throughout the period to confirm the total absence of infusoria. At the end of this period of either 14 or 21 days, a considerable quantity of rumen contents of an infected animal was expelled from a glass tube into the throat of the goat. After a week the rumen was always teeming with infusorian life. Roughly calculated, in one goat there were about 2,000,000 infusoria per c.c. The amount of food was not changed during this period. At the end of an intervening period of ten days, daily collections of feces and urine were again initiated and carried on throughout the infected period.

The metabolism crates in which the goats were kept during the digestion trials were designed with four purposes in mind; maximum comfort for the goat, elimination of any loss of feed in the process of feeding, collection of all the feces, and collection of the urine separate from the feces and without loss. The general construction is shown in figures 2 and 3, but certain special points will be mentioned. The crate was made sufficiently large so that it permitted the animal to turn around in it: height, 30 inches; length, 48 inches; width, 21 inches. It will be noted (fig. 2) that the stanchion through which the goat thrust its head in order to eat from the feed box was somewhat V-shaped. The head could enter at the top, but could not be withdrawn at the bottom. This prevented the animal from scattering feed within his crate and mixing it with its own excrement. The floor under the crate was of cement so that any particles of hay or grain which fell from the feed-box might be readily swept up and refed.

The collection of the feces unmixed with urine was accomplished by the use of screens. The floor of the crate was of  $\frac{1}{2}$  inch mesh hardware



cloth, through the openings of which the pellets passed as they dropped down onto a copper screen covering a galvanized iron tray to be described presently (fig. 3). From this screen they were scraped with a large piece of tin into evaporating pans. Pellets which failed to pass through the hardware cloth were picked up. The sides of the crate part way up from the bottom, as shown in the figure, were covered with tin to prevent pellets dropping to the floor. Any which fell onto the cement floor despite these precautions were swept up and saved.

Urine collection was effected by means of a galvanized iron tray beneath the entire floor of the crate. It was supported by cross-pieces between the legs of the crate, and could be withdrawn at one end. The lid of this tray was composed of copper screen framed in galvanized iron, which was bent so that it fit down over the edges of the tray itself. The screen caught the fecal pellets, but permitted the urine to pass through. The upper edge of the tray supporting the lid was horizontal, but the tray was so constructed that it was  $\frac{1}{2}$  inch deep at one end and  $2\frac{1}{4}$  in. at the other. This made an incline down which the liquid flowed toward one end. Here was a small spout leading from the bottom. An enamel pan beneath held the urine which passed through this spout. Just after the daily collection of feces and urine, the screen and tray were carefully washed down with distilled water from a sprinkling can, and the wash water was preserved with the urine.

The foregoing account of our methods shows certain points which we wish to emphasize. Each goat, except No. 1, received a uniform ration of hay and grain during both periods. These feeds were each thoroughly mixed, weighed out, and sacked beforehand; and the same amounts were fed daily during the two periods of equal length. Daily collections were made of feces and urine, and these materials were treated so as to reduce changes from bacterial action to a minimum. Later the fecal pellets were ground into a fine powder, and those for each period were thoroughly mixed. The urine was all put into one large glass bottle and thoroughly mixed. Samples of hay and grain mixture for chemical analysis were obtained by saving a handful now and then at the time they were weighed out. Both the hay and grain thus saved were thoroughly mixed and weighed. In this manner aliquot samples thoroughly representative of both hay and grain were obtained for chemical analyses. Also, it should be stressed that comparisons were not made between different goats, but the results secured in different trials with the same animals were compared.

As stated in the foregoing paragraphs, we used trial periods of 14 days each in the case of two of the animals and of 21 days in the case of the other two. These intervals are somewhat longer than the minimum usually considered to be sufficient in digestion trials of this nature. Our information relative to the length of trials was gathered from the recommendations of Forbes and Grindley (1923) for the sub-committee on animal nutrition of the National Research Council, the observations of Schneider and Ellenberger (1927), and previous experience. It has previously been shown that it is impracticable to attempt to mark goat feces by the feeding of charcoal or carmine, so no attempt was made to mark them in this experiment.



## FIGURE 2.

Structure of anterior end of metabolism crate. Stanchion can be closed, to hold goat's head for obtaining sample of rumen contents, by shifting bar *c* from hole *a* to hole *b* and bolting, the bar pivoting on bolt *h*.

## FIGURE 3.

Showing construction of metabolism crate. Coarse mesh is hardware cloth of  $\frac{1}{2}$  inch mesh. Fine mesh is copper screen.

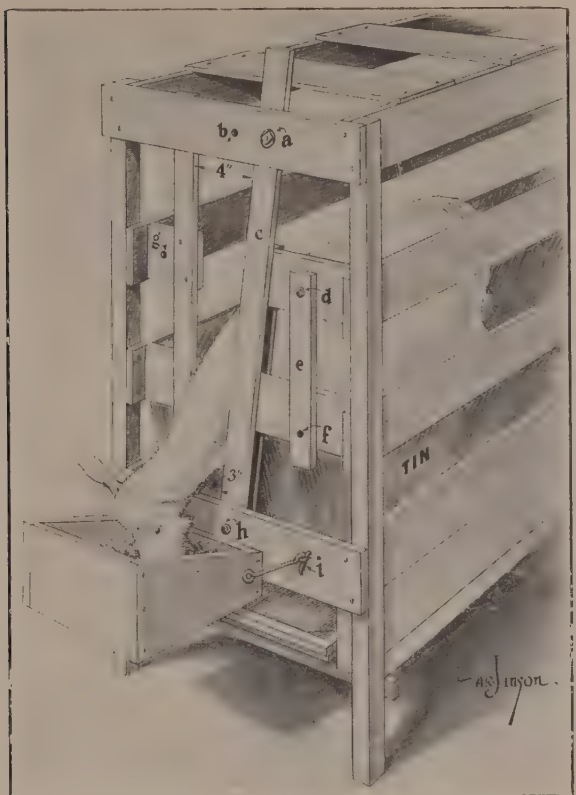
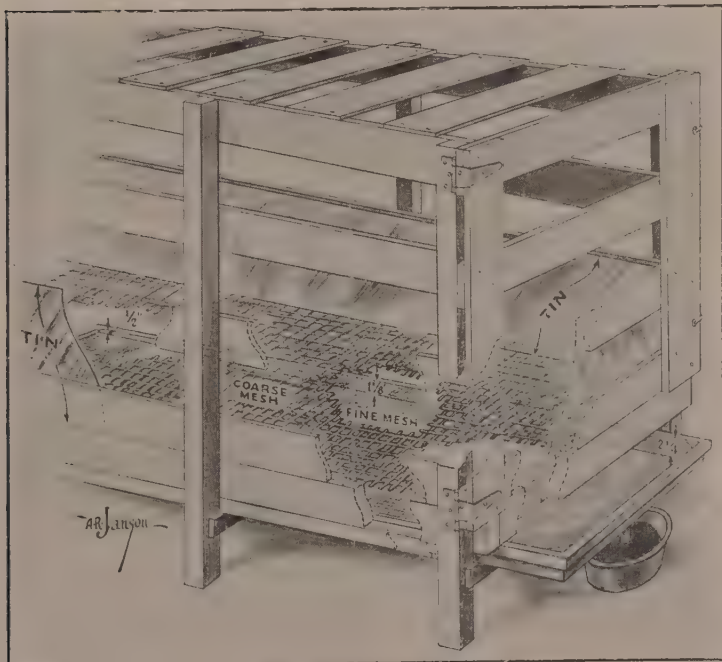


FIGURE 2.





## NON-INFUSORIAN LIFE OF THE RUMEN

Any adequate presentation of the various factors that obtained in our experiments would require at least brief discussion of the organisms which were present in the rumen of our animals. During the infusoria-free period, as the name implies, there were no infusoria present in the rumen contents of our animals. This was not taken for granted, but the fact was verified by frequent microscopic examinations of samples of rumen contents.

The flagellates *Trichomonas ruminantium* and *Callimastix frontalis* were present to a greater or less degree in our animals during both periods. It seems to be impossible to permanently exclude them, although no other flagellates appeared during the infusoria-free period. It must be emphasized, however, that *Callimastix* was never abundant and *Trichomonas* did not at any time abound in the overwhelming numbers that we sometimes find in the stools of animals. Sometimes flagellates were so rare that an average of one flagellate per field was observed in the fraction of a drop under the low power of the microscope. Never were there more than an average of eight or ten in such an area. We feel that the flagellate protozoa were a negligible factor, for at no time was their mass anything like more than a very small fraction of the mass of infusoria during the infected period. According to certain authorities, there may be as many as 2,000,000 infusoria per c.c. of rumen contents; and when we consider the additional facts that the volume of a flagellate is only a small fraction of that of even the smallest of these infusoria, and that they are not ingestors of plant fiber to any extent, as are certain of the infusoria, it is evident that the flagellates present during the infusoria-free period could not have assumed any of the hypothetical rôles attributed to the infusoria.

Bacteria were by far the most numerous organisms present during both periods. No special study was made of them beyond the observations that they were of the rod and coccus types and that Gram's negative greatly preponderated over the Gram's positive. In all four of the animals during both periods there was present in large numbers a rather distinctive, large, Gram's negative, rod-shaped bacterium with rounded ends (fig. 4). Internally it showed what appeared to be transverse partitions. A further peculiarity of this organism was that it stained blue with iodine some time after the animal had received its grain ration, showing that it had absorbed starch.

Did the defaunation process alter the character of the bacterial flora of the rumen? In view of the fact that there is considerable evidence that cellulose digestion in herbivores is accomplished through the agency of bacteria, it was important that this possibility be kept in mind. So little is known about the rumen flora, that it would have been practically impossible for us to have determined whether or not the copper sulphate treatment had produced any change in the cellulose digesting bacterial population. It must be admitted, however, that the large bacterium discussed in the preceding paragraph always disappeared from the rumen simultaneously with the infusoria, and did not return spontaneously, at least in the time allowed. Thus there was a possibility that the character of the bacterial population was considerably changed by the defaunation treatment.



An attempt was made to insure a restoration of the normal flora by re-inoculation. This we aimed to accomplish in the following manner: several c.c. of liquid rumen content were taken from a normal goat. This was poured into a test tube and taken out into the cold air or put into a refrigerator for a few minutes. The cooling immobilized the infusoria, and most of them settled to the bottom. A small amount of the remaining bacterial suspension was examined drop by drop under the low power of the microscope for infusoria. Those drops which were free of the protozoa were saved and drawn up into a fine pipette. The contents of this pipette were then injected down the esophagus of the defaunated animal. In all cases we succeeded in effecting the return, within a week or more, of the large rod-like bacterium discussed above, without infecting the defaunated goats



Figure 4. Large bacillus with appearance of transverse partitions and rounded ends. At upper right is one containing what is possibly a spore.

with infusoria. If any other forms of bacteria had been eliminated they were thus afforded at least an equal opportunity to return. Thus it is quite likely that the floral equilibrium was left not seriously disturbed for the infusoria-free period of the digestion trials.

#### THE INFUSORIAN FAUNA

From the tables of Dogiel (1927, pp. 231-234) we learn that there had been recorded from the goat up to that time 19 species of the Ophryoscolecidae. These are divided as follows among the genera of this family: *Entodinium*, 7; *Diplodinium* (including all subgenera), 10; *Ophryoscolex*, 2. Other ciliates which we might expect to find at times in goats are *Isotricha*, *Dasytricha*, *Buetschlia*, or even *Charon* and *Blepharocorys*. Becker and Talbott (1927) were inclined to the belief, implied in several places in their paper, that the different form types of Ophryoscolecidae did not necessarily represent species, or even varieties, and that they might be merely transitional forms. Consequently, they adapted a policy of "lumping" as con-

trusted with Dogiel's policy of "splitting." We now feel that Dogiel has adopted the correct course, for our experience has taught us that when a defaunated animal is inoculated with certain known types, the ensuing fauna developing in its rumen do not offer any striking modifications of the original types. For example, as discussed later, the rumen of a goat into which was introduced a single species, *Entodinium simplex* (?), became infected with types like those in the original inoculation. Other goats inoculated with known species became the hosts of these same specific types.

The importance of recording the species of infusoria present in the rumen during the infected periods of our experiments is apparent. Most of the species of Ophryoscolecidae were determined according to their degree of correspondence with the descriptions of Dogiel (1927). We have not employed sub-generic names. The following table shows the species with which each goat used in the trials was infected.

TABLE 1. *Species of Infusoria Present in Rumens of Each Goat.*

- Goat 1. *Entodinium longinucleatum*, *E. simplex*, *E. minimum*, *D. caudatum hamatum*.
- Goat 2. *Entodinium simplex* (?).
- Goat 3. *Entodinium simplex*, *E. vorax*, *E. minimum*, *E. caudatum*, *Diplodinium caudatum hamatum*, *D. bursa* (See Becker and Talbott for description), *D. multivesiculatum*, and *Iso-tricha intestinalis*.
- Goat 4. Same as Goat 3.

It will be noted that Goat 2 appeared to have a pure infection of *Entodinium simplex*. The morphology of this ciliate did not check exactly with Dogiel's description, especially in regard to the shape and location of the macronucleus and the nature of the food. Considerable plant fiber, chlorophyll, and starch grains were often found in the endoplasm of our strain. The pure strain was obtained from another goat which originally had a number of species of infusoria. Several attempts to defaunate with acetic acid had eliminated all species but this one, which persisted in coming back.

Perhaps a few words here regarding the life-history and transmission of the ruminant infusoria would not be out of place. Geographically, they seem to be universally distributed. In the rumen they multiply in enormous numbers by binary fission. No authentic cysts of these protozoa have ever been observed, but some of the earlier workers believed the source of the infection to be cysts or resistant forms on the hay. The observation that the infusoria first appeared in lambs at the time they began to eat hay contributed to this belief. Eberlein (1895) and Dogiel (1927) have observed that when different species of ruminants were thrown into proximity each species is likely to acquire a mixed fauna. While this type of observation does not bear exactly upon the question of the existence of cysts, it implies that there is no universal distribution of resistant forms on herbage. Otherwise, a mixing of the faunas would occur without the animals being brought into proximity. Becker and Hsiung (1929) have shown that in all probability cysts do not exist, and that the infection is spread by contamination of feed with trophozoites in the saliva from the mouth. That the fauna is at least somewhat non-specific was shown by these same workers, for they were able to infect defaunated goats with

infusoria from cattle, and sheep with those from goats. Eberlein (1895) had previously observed that wild ruminants brought into a zoo acquired the fauna of the domestic ruminants of the region. Dogiel (1927) also made observations contributing to the idea that there is no rigid host-specificity, although there is evidence for some degree of it.

Weill (1929) has recently made the novel and interesting suggestion that certain insects may have a rôle in the transmission of ruminant infusoria. This idea is based upon his discovery of an *Isotricha* in the intestine of a cockroach. There are a number of reasons why such a method of transmission seems to us to be improbable, but the problem is now before us and demands more investigation.

No satisfactory method of cultivating these protozoa has been developed as yet. Among those who have made efforts in this direction are Coste (1864), Edwards (1864), Pouchet (1864), Schuberg (1888), Certes (1889), Günther (1899), Fiebiger (1923), and Becker and Talbott (1927). Knoth (1928), with his hay cultures, has come the nearest of all to success. His method was unique in that he replaced the air in the suction flasks containing his culture medium and infusoria with a gaseous mixture consisting of 65 per cent  $\text{CO}_2$  and 35 per cent methane, which he found to be the proportions present in the normal rumen. Magnesium oxide was added to the medium to neutralize the acids produced. The pH was kept as near 6.8 as possible. Some of his infusoria lived for 107 hours.

#### CHEMICAL ANALYSIS

Moisture content of feeds and feces was determined by heating the samples to a constant weight in a Freas vacuum oven at a temperature of  $60^\circ\text{C}$  and in a vacuum of 26 to 29 in. of mercury.

Ash, crude protein, ether extract, and pentosans were determined according to the Official Methods of Analysis of the Association of Official Agricultural Chemists (1925).

Alpha, true or normal cellulose (Cross and Bevan, 1916) was determined by treating the samples with four per cent  $\text{NaOH}$  at 180 pounds pressure for one hour according to the method of Mehta (1925).

The hemicelluloses were determined by hydrolysing the material with 2.5 per cent  $\text{H}_2\text{SO}_4$  (by weight) for two hours. After filtration, neutralization, clarification with neutral lead acetate, and removal of excess lead with dry  $\text{NaHCO}_3$ , the reducing sugars in the filtrates were determined as dextrose by the method of Quisumbing (1921).

Crude fiber was determined by the Morgan O. Sweeney method as modified by Kennedy (1912).

Tables 2 to 9, inclusive, present the percentage constituents of feeds and excreta, the intake and excretion of nutrients, and the amounts of each nutrient digested by each goat during each period. Attention is called particularly to the fact that the percentage of nitrogen or protein in the feces of all animals was greater in the case of the feces voided during the first period. The total amount of nitrogen excreted in the urine was, however, greater for each goat during the second period. But in the case of Goat 1 more feed was fed during the second period.

Table 10 shows, for both periods, the coefficients of digestibility of all nutrients, the nitrogen balance, the partition of the nitrogen excretion, the nutritive ratio of the rations, and the weights of the goats.



It was originally planned to include in the experiment the ash, calcium, magnesium, and phosphorus balances, but it was discovered that the distilled water available had a deleterious effect upon the growth and multiplication of the infusoria in the rumen when given to the animals for drink. Qualitative chemical tests upon the slight residues obtained by evaporating large quantities of the distilled water used failed to show the presence of copper or any other metals which might conceivably cause this deleterious effect. The residue consisted largely of silicate salts derived probably from the storage containers or glassware. Therefore, tap water was fed to the goats; and since under the conditions of the experiment ash and mineral balances were not considered essential to the purposes and ends in view, no attempt was made either to analyze the water or to record the amounts drunk by the animals.

While the chemical and biological literature contains hundreds of papers, which record the results of digestion and metabolism trials with steers, dairy cattle, pigs and sheep, there are but few experiments published which can be used for close comparisons. A search through the extensive literature on the metabolism and digestion of ruminants reveals but few cases in which goats were used and results other than merely the mineral balances were published. Most of this material is older work, and was planned to determine the digestibility of single roughages and feeds, or to show how the addition of certain feeds, chemicals, or supplements modified the digestion and utilization of the feed, or the milk production of the animal. The researches of Emery and Kilgore (1892, 1894), Fingerling (1911 (a) and (b)), Morgen, Beger and Westhauser (1909, 1910), and Snyder (1893) are typical, but because of the differences in the character of the rations used, and the procedures followed, the results obtained can not be compared too directly with the results we have obtained upon the coefficients of digestibility of the nutrients in our ration. We do find, however, that our digestion coefficients do fall well within the limits established by these early investigations, wherever the rations and animals are at all comparable, for the nutrients studied by them. A more recent publication by Hunt, Winter, Schulz and Miller (1923) offers a somewhat closer basis of comparison, especially during those periods in which dry feeds were fed. We were unable to find any records of previous reports upon the digestion coefficients of alpha cellulose, pentosans and hemicelluloses where goats were used as the experimental animals.

Considering the fact that the goats used in this experiment were of varying breeds, sizes and ages, the coefficients of digestibility of the nutrients studied present a normal agreement. The maximum variation in the percentages of digestibility was 16.07 per cent, shown, as would be expected from the results of other investigators, by the ether extract. The variation in the digestion coefficients of the cellulose materials varied from 7.30 per cent for alpha cellulose, and 5.85 per cent for hemicellulose to 1.83 per cent for the pentosans. There was a normal variation of 2.97 per cent in the digestibility of the protein, of 3.54 per cent, and 3.41 per cent in the digestibility of the nitrogen-free extract and total dry matter, respectively.

The variation in the ability to digest crude fiber and hemicellulose as shown by Goat 1 in periods I and II was greater than that exhibited by the other goats in comparison with each other, or with Goat 1. This may be



due to the fact that Goat 1 was probably somewhat under-fed during the first period.

The coefficients of digestibility of all nutrients, as determined by us, compare favorably with those determined by other investigators, with the possible exception that our figures for the digestibility of crude fiber are somewhat lower than those reported by some investigators using goats in their studies. This difference in results is possibly due to the fact that the alfalfa hay used by us, while containing a good percentage of protein (tables 2 to 9) was somewhat dark in color, contained a rather high percentage of stems, and had undoubtedly weathered slightly. It was, however, the best hay available at the time.

All of the goats used in this experiment stored some nitrogen, the daily storage varying from 0.37 grams by Goat 3 in period II, to 4.84 grams by Goat 2 in period I. No attempt was made to estimate the nitrogen loss from the body through perspiration, or by the collection of brushings. These positive nitrogen balances, while small, indicate that the animals, though apparently receiving, designedly, rather meager rations, and in some cases losing weight during the progress of the experiment, were nevertheless in a fair state of nutrition so far as their protein requirements were concerned. Only Goat 2, during period I, excreted a greater percentage of her total excreted nitrogen in the feces than in the urine. This goat may have been underfed before being placed on experiment, stored nitrogen consistently while on experiment, and probably could have received more feed, without danger of going stale or refusing feed.

#### THE EFFECTS OF INFUSORIA ON DIGESTION

A perusal of table 10 indicates that on the whole the presence or absence of infusoria, in the rumens of these goats had but little demonstrated effect upon the coefficients of digestibility of the nutrients. Goats 1 and 2, young, immature, and growing before and after being placed on experiment, digested slightly greater percentages of all nutrients (with the exception of pentosan in the case of Goat 2, and ether extract in the cases of both goats) while they were infected with infusoria. Goat 3 digested smaller percentages of all nutrients, except protein and ether extract, while infected with protozoa, while Goat 4 digested greater percentages of all nutrients except crude fiber, pentosan and alpha cellulose while thus infected.

Larger percentages of dry matter, hemicellulose, and nitrogen-free extract were digested by three out of four goats while infected with infusoria. Only two out of the four goats increased their percentage digestion of ether extract, crude fiber and alpha cellulose when thus infected; and only one, Goat 1, digested a higher percentage of pentosan material while harboring these infusoria. Since certain previously cited publications have indicated the possibility that infusoria have some part in the digestion of cellulose in ruminants, it was in the digestion of the cellulose materials that we expected to find the greatest differences. Goat 1 was the only one of our experimental animals, however, which digested appreciably greater amounts of the cellulose material while infected with infusoria, and even these differences in digestive ability are probably too small to be of any practical significance. At any rate, any apparent significance which they might have is probably nullified by the fact that the more mature

goats, 3 and 4, digested with the one exception of hemicellulose, greater percentages of crude fiber, alpha cellulose and hemicellulose in the periods when they were free from infusorial infection.

While it is true that the average figures for the coefficients of digestibility for the four goats are slightly greater, with the exception of the figure for pentosan, in those periods when the goats were infected with infusoria, it may be seen that these average figures are in most cases greater because of the results obtained from one goat out of the four. Our only complete unanimity between the four goats was obtained in the digestion of protein, all goats digesting slightly more protein during those periods when they were infected with infusoria. These results on the digestion of protein may offer some slight support to theories propounded to the effect that the infusoria may manufacture proteins from non-protein nitrogen compounds, or otherwise exert a protein sparing action in the paunch of ruminants. Since, however, the largest increase present, obtained with Goat 1, was less than five per cent of the total percentage of protein digested, and since an increase in protein storage followed infection with infusoria in only two goats out of the four, we feel that our results on protein digestion thus far offer little more than a possible encouragement to the further study of the relation between infusoria and the digestion of protein in ruminants. Since (tables 2 to 9) the percentages of protein present in the feces were found to be greater in the feces voided by all goats during the first period, when the goats were infusoria free, it may seem that the protein constituting the bodies of the infusoria may have been more easily digested than that of the plant foods in the ration fed. These differences in the percentages of protein present in the feces voided during the two periods by each goat are possibly small enough to be accounted for by the bodies of the infusoria; and if so, the differences in the digestibility coefficients for the proteins during the two periods might not lend support to any theory of protein sparing action by the infusoria which does not first presuppose the changing of plant nitrogen into infusorial proteins. Thus it would be possible from our results to deduce the theory that the infusoria may be benign and very slightly useful parasites. Any further studies should probably be made upon the rumen contents by means of fistulae rather than upon the feces and urine, since it is quite possible that the bacteria in the intestinal tract obliterate at least some of the changes in the protein metabolism which may have been initiated by infusoria in the rumen.

#### THE EFFECT OF THE PROTOZOA ON pH AND ON PUTREFACTION

The pH of rumen contents of Goat 1 was taken on two different days during each period by the color method. They were as follows: infusoria free period, 7.2 and 7.4; infected period, 7.4 and 7.2. Tests on some of the other goats during both periods gave similar results. At any rate, the pH is not affected by the protozoa.

It seems untenable that the infusoria may aid their host in keeping down the growth of microorganisms which, if unchecked, might become injurious. No untoward effects on the health have been observed in these and other goats. One of these was free of infusoria for over six months. Likewise, the infusoria-free lambs of Becker and Everett (1929) showed a

healthy rate of growth. The Ehrlich test for putrefaction (indol) made several times on rumen contents of Goat 1 during the infusoria-free period gave only negative results. Consequently, we cannot ascribe to the infusoria any rôle in keeping down the growth of injurious microorganisms of the nature of Schizomycetes.

#### DISCUSSION AND SUMMARY

Let us discuss briefly the bearing of the results of our experiments and observations upon each of the previously held views regarding the relationships between the rumen infusoria of ruminants and their hosts.

That the infusoria convert plant substances into the animal substance of their own bodies, and that these in turn are sacrificed to the digestive juices in the stomach and intestine, are matters of common observation. That the host animal derives any substantial benefit from possible activities of the infusoria, such as either synthesizing proteins from simpler nitrogenous substances as amides or converting plant protein into a more easily digestible animal protein, as discussed above, is doubtful. Mangold (1929) has recently discussed the results of the work of Ferber (1928), who failed to secure any consistent increase in the numbers of infusoria by adding urea and ammonium acetate to protein poor and carbohydrate rich feeds. Our results are consistent with these findings, but certainly not with these authors' conclusions that "Offenbar erleichtern die Panseninfusorien dem Wiederkäuer die Eiweiss-Ausnutzung indem sie das schwerer verdauliche, pflanzliche Eiweiss seines Futters zunächst in das leichter verdauliche Eiweiss ihrer eigenen Leibessubstanz umwandeln." Neither are our results nor the recently reported results of Becker and Everett (1929), in which infusoria-free lambs made somewhat greater gains in weight than infected lambs, lend support to such a hypothesis.

If the slightly lower protein content of the feces in our infected animals represents an actuality, and is not due to chance, it does not necessarily signify any advantage to the animal, for it must be remembered that the infusoria themselves metabolize a certain amount of protein. Thus it is even possible that they may represent a liability rather than an asset in the economy of the animal. If this should prove to be true, the protozoa could be regarded as harmful parasites only in the rôle of "food-robbers."

It should be mentioned here that protein synthesis from amides in the paunch of ruminants is by no means proved, although the early work of Zuntz (1891, 1913), Hagemann (1891), and others (Müller, Hansen) seemed to supply much evidence for it. Mangold (1929) has discussed some of the more recent negative results. There still will be time for determining whether it is the protozoa or the bacteria that are responsible for protein synthesis after it is positively determined whether or not such synthesis actually takes place.

As discussed above, the infusoria are not to be regarded as a natural restraining influence upon the inordinate multiplication of the Schizomycetes in the rumen. Many of these infusoria eat some bacteria, but activities of this nature are of no noticeable consequence on the health of the host.

As regards cellulose digestion, our figures for the amounts of it digested in infusoria-free and infected animals show little difference. We wish to make one point clear—we do not wish to commit ourselves at present on the controversial point as to whether or not some or all of the Ophryoscolecidae



can digest cellulose. Our problem is one of host-parasite relationships rather than one of the physiology of digestion inside a protozoon. According to Trier (1926), these infusoria can digest starch, converting it into glycogen. According to Ferber (1928), they can ingest fat, and digest it with the aid of certain bacteria. But we do not know for sure if they can digest cellulose (*Vide ut supra*). Our contribution consists in the simple discovery that cellulose digestion in the host is neither due to, nor assisted materially by the infusoria.

The suggestion of Trier (1928) that there may be a symbiosis between infusoria and cellulose-digesting bacteria is likewise untenable, for cellulose digestion took place in animals free from infusoria for weeks.

Incidentally, one sometimes hears that it is still uncertain that animals can digest true cellulose. This doubt was echoed by Cleveland (1925). We have found that the goat digests crude fiber, hemicelluloses, alpha cellulose, and pentosans in considerable amounts (see tables).

The suggestions, discussed above, that the infusoria may be valuable in purely mechanical ways (thorough mixing and trituration of the rumen contents) likewise seem to be unsubstantiated by our experiences. Digestion seems to have progressed as well in an infusoria-free goat as in an infected one. Likewise, there were no rumen impactions or complications due to mechanical causes. It is true that these goats were not permitted to engorge to capacity. The lambs of Becker and Everett (1929), however, did have this privilege. These animals have not experienced any rumen difficulties to date.

After eliminating serious consideration of all other hypotheses, there still remains the view that the rumen infusoria are mere commensals. We feel more closely committed to this one than to any of the others.

#### CONCLUSIONS

1. The infusoria of the ruminant stomach are of no substantial value to their host in converting plant proteins into more easily digestible animal protoplasm.
2. It is doubtful if they can convert amides or protein derivatives into the protein of their own bodies.
3. They are of no appreciable value to their host in the digestion of carbohydrates.
4. There is no demonstrable symbiosis between the infusoria and cellulose-digesting bacteria of the rumen.
5. They serve no significantly useful purpose in suppressing the numbers of *Schizomycetes* in the rumen.
6. They apparently perform no mechanical services of any value by way of mixing, trituration, or soaking the roughage in the rumen.
7. They exert no effect upon the hydrogen-ion concentration of the rumen.
8. The physical well-being of the host is not perceptibly improved by removal of this fauna.
9. Since there is no reason for regarding these infusoria either as constituting a useful endofauna or true parasites, we must consign them definitely, for the present, to the status of mere commensals.
10. It remains to be seen whether or not they may be "food-robbers" to a limited extent.



## LITERATURE

## (A) Bibliography to the stomach protozoa of domesticated ruminants.

AWERINZEW, S., AND R. METAFOWA.

1914. Material zur Kenntnis der Infusorien aus dem Magen der Wiederkäuer. *Arch. f. Prot.*, **33**:109-118.

BECKER, E. R.

1929. Methods of rendering the rumen and reticulum of ruminants free from their normal infusorian fauna. *Proc. Natl. Acad. Sci.*, **15**:435-438.

BECKER, E. R., AND RALPH C. EVERETT.

1929. Progress report on weight increases in lambs with and without rumen infusoria. *Proc. Natl. Acad. Sci.*, **15**:683-684.

BECKER, E. R., AND W. W. FRYE.

1927. Some protozoa found in the feces of cattle. *Proc. Ia. Acad. Sci.*, **34**:331-333.

BECKER, E. R., AND T. S. HSIUNG.

1929. The method by which ruminants acquire their fauna of infusoria, and remarks concerning experiments on the host-specificity of these protozoa. *Proc. Natl. Acad. Sci.*, **15**:684-690.

BECKER, E. R., J. A. SCHULZ AND M. A. EMMERSON.

1929. A comparative study of the digestion of proteins and carbohydrates in goats during infusoria-free and infected periods. *Proc. Natl. Acad. Sci.*, **15**:691-693.

BECKER, E. R., AND M. TALBOTT.

1927. The protozoan fauna of the rumen and reticulum of American cattle. *Iowa State College Jour. Sci.*, **1**:345-373.

BIEDERMANN, W.

1911. Die Aufnahme, Verarbeitung und Assimilation der Nahrung in Handb. d. vgl. Physiol. Hrsg. H. Winterstein, Bd. 2. Jena.

BRANDT, H.

1909. Beiträge zur Biologie der Infusorien in Digestiontractus der Herbivoren. Inaug. Diss., Bern.

BRAUNE, R.

1913. Untersuchungen über die im Wiederkäuermagen vorkommenden Protozoen. *Arch. f. Prot.*, **32**:111-170.

BUISSON, J.

1923. *Les Infusoires Ciliés du Tube Digestif de l'Homme et des Mammifères*. Paris.

BUNDLE, A.

1895. Ciliate Infusorien im Cöcum des Pferdes. *Zeitschr. f. wiss. Zool.*, **60**:284-350.

CAMPBELL, A. S.

1929. The Structure of *Isotricha prostoma*. *Arch. f. Prot.*, **66**:331-339.

CERTES, A.

1880. Sur la glycogénèse chez les infusoires. *Comp. Rend. Acad. Sci.*, Paris, **90**:77-80.

1889. Note sur les microorganismes de la panse des ruminants. *Bull. Soc. Zool. France*, **14**:70-73; also, *Jour. de Microgr.*, **13**:277-279.

CLEVELAND, L. R.

1924. The physiological and symbiotic relationships between the intestinal protozoa of termites and their host, with special reference to *Reticulotermes flavipes* Kollar. *Biol. Bull.*, **46**:177-225.

- COLIN, G.  
1854. *Traité de Physiologie comparée des Animaux domestiques*. Paris.
- COSTE, M.  
1864. *Developpement du Infusoires ciliés dans une Maceration de Foin*. C. R. Acad. Sci., **59**:149-155.
- CRAWLEY, H.  
1923. Evolution in the ciliate family Ophryosecolecidae. *Proc. Acad. Nat. Sci., Phila.*, **75**:393-412.
- CUNHA, A. M. DA  
1914. *Sore os cilados do estomago dos ruminants domesticos do Brasil*. Mem. Inst. Osw. Cruz., **6**:58-68.
- DOPFLEIN, F.  
1916. *Lehrbuch der Protozoenkunde*. Vierte Auflage. Jena.
- DOGIEL, V.  
1923. Cellulose als Bestandteil des Skelettes bei einigen Infusorien. *Biol. Zentralbl.*, **43**:289-291.  
1925. Ueber die Art der Nahrung und der Nahrungsaufnahme bei den im Darne der Huftiere parasitierenden Infusorien. (Abstract in German, remainder in Russian.) *Trav. Soc. Nat. Petrograd, Sect. Zool. and Physiol.*, **54**:67-93.  
1926. Une nouvelle espèce du genre *Blepharocorys*, *B. bovis*, habitant l'estomac du Boeuf. *Ann. Parasit. hum. et compar.*, **4**:61-64.  
1927. *Monographie der Familie Ophryosecolecidae*. *Arch. f. Prot.*, **59**:1-288.  
1928. Über die Conjugation von *Buetschlii parva*. *Arch. f. Prot.*, **62**:80-95.
- DOGIEL, V., UND T. FEDOROWA.  
1925. Über den Bau und die Funktion des innern Skeletts der Ophryosecoleciden. *Zool. Anz.*, **62**:97-107.
- EEBERLEIN, R.  
1895. Über die im Wiederkäuermangen ciliaten Infusorien. *Zeit. f. Wiss. Zool.*, **59**:233-304.
- EDWARDS, M.  
1864. *Remarques de M. Milne Edwards a l'occasion de la précédente Communication: Developpement des Infusoires cilies une maceration de Foin*. C. R. Acad. Sci., **59**:155-156.
- FANTHAM, H. B.  
1922. Some parasitic protozoa found in South Africa. V. *So. African Jour. Sci.*, **19**:332-339.  
1926. Some parasitic protozoa found in South Africa. IX. *So. African Jour. Sci.*, **23**:560-570.
- FERBER, K. E.  
1928. Die Zahl und Masse der Infusorien im Pansen und ihre Bedeutung für den Eiweissaufbau beim Wiederkäuer. *Zeitschr. f. Tierzücht und Züchtungsbiologie*, **12**:31-63.  
1929. Die Veränderung der Infusorienzahl im Pansen der Wiederkäuer im Zusammenhang mit den Veränderung des Eiweissumsatzes. *Ibid.*, **15**:375-390.
- FERBER, K. E., UND T. WINOGRADOWA-FEDOROWA.  
1929. Zählung und Teilsquote der Infusorien im Pansen der Wiederkäuer. *Biol. Zentralbl.*, **49**:321-328.
- FIEBIGER, J.  
1923. Die tierischen Parasiten der Haus und Nutztiere sowie des Menschen. Leipzig. pp. 124-133.

## FIORENTINI, A.

1889. Intorno ai protisti dello stomaco dei bovini. Pavia.  
 1890(a). Intorno ai protisti dello stomaco dei bovidi. Boll. Sci. (Maggi). 11:87-91.  
 1890(b). Sur les protistes de l'estomac des bovidi. Jour. de Microg., 14:23-28; 79-83; 178-183.  
 1892. Antwort des Dr. Angelo Fiorentini dem Dr. Schuberg. Centralbl. f. Bakt. u. Parasit., 11:758-760.

## FONSECA, O. O. R. DA.

1916. Estudos sobre os flagellados parasitos dos memmiferos do Brazil. Mem. Inst. Os. Cruz, 8:5-40.

## GRUBY et DELAFOND.

1843. Recherches sur des animalcules se développant en grand nombre dans l'estomac et dans les intestins pendant la digestion des animaux herbivores et carnivores. Compt. Rend. Acad. Sci. (Paris), 17:1304-1308. Also, Recueil de Médecine Vétérinaire Pratique, 20:859-866.

## GÜNTHER, A.

1899. Untersuchungen über die im Magen unserer Hauswiederkäuer vorkommenden Wimperinfusorien. Zeitschr. f. wiss. Zool., 65:529-572.  
 1900. Weitere Beiträge zur Kenntnis des feineren Baues einiger Infusorien aus dem Wiederkäuermagen und dem Coecum des Pferdes. Zeitsch. f. wiss. Zool., 67:640-662.

## JAMESON, A. P.

1925. A new ciliate, *Charon ventriculi*, n. g., n. sp., from the stomach of ruminants. Parasitol., 17:403-405.

## KNOTH, M.

1928. Neue Versuche zur Züchtung der im Pansen von Wiederkäuern lebenden Ophryoseoléciden (Ciliaten). Zeitschr. f. wiss. Biol. Abt. F, 1:262-282.

## KLEIN, W.

1926. (Oral communication to Trier) Cf. Trier, 1926, 323-324.

## LIEBETANZ, E.

1910. Die parasitischen Protozoen der Wiederkäuermagens. Arc. f. Prot., 19:19-80.

## LIST, A.

1885. Untersuchungen über die in und auf dem Körper des gesunden Schafes vorkommenden nieren Pilze. Inaug. Diss., Leipsiz.

## MANGOLD, E.

1929. Neuere Forschungen über Ernährung und Verdauung der Wiederkäuer. An address to the Landw. Club, Mannheim.

## MANGOLD, E., UND C. SCHMITT-KRAHMER.

1927. Die Stickstoffverteilung im Pansen der Wiederkäuer bei Fütterung und Hunger und ihre Beziehung zu den Pansen-Infusorien. Biochem. Zeitschr., 191:411-422.

## MEYER, W.

1927. Vergleichende mikroskopische Untersuchungen ueber die Verdauung der Kleberzellen, . . . Zeitschr. f. vrgl. Physiol., 6:402-430.

## POUCHET, F. A.

1864. Embryogénie des Infusoires ciliés. Response aux Observations de M. Coste. C. R. Acad. Sci., 59:276-283.

## REICHENOW, E.

1920. Die Wiederkäuerinfusorien verwandte Formen aus Gorilla und Schimpanse. Arc. f. Prot., 41:1-33.  
 1927. Lehrbuch der Protozoenkunde begründet von F. Doflein. 1. Teil. Jena.

- SCHEUNERT, A.  
1924. Verdauung der Wirbeltiere in Oppenheimer, C. *Handbuch d. Biochem. d. Menschen u. d. Tiere*.
- SCHEUNERT, A., UND M. SCHIEBLICH.  
1927. In Bethe, Bergmann, Embden, und Ellinger. *Handb. norm. u. path. Phys. Berlin*.
- SCHUBERG, A.  
1888. Die Protozoen des Wiederkäuermagens. *Zool. Jahrb.*, 3:365-418.  
1891. Ueber einige Organisations-verhältnisse der Infusorien des Wiederkäuermagens. *Sitzber. d. Phys. Med. Ges. Würzburg*, 1891:122-137.  
1892. Bemerkungen zu den Untersuchungen des Herrn Dr. Angelo Fiorentini über die Protozoen Wiederkäuermagens. *Centralbl. f. Bakt. u. Parasitenk.*, 11:760-761.
- SCHULZE, P.  
1924. Der Nachweis und die Verbreitung des Chitins mit einem Anhang über das Komplizierte Verdauungssystem der Ophryoscoleciden. *Zeitschr. f. Morph. u. Ökol.*, 2:643-666.  
1927. Noch einmal die "skelettplatten" der Ophryoscoleciden. *Zeitschr. f. Morph. u. Ökol.*, 7:678-689.
- SCHUURMAN, JOHANNA F. M.  
1926. Some remarks on the geographical distribution of certain ciliates found in the digestive tracts of ruminants. *So. African Jour. Sci.*, 23:571-574.
- SCHWARZ, C.  
1925. Die ernährungs physiologische Bedeutung der Mikroorganismen in den Vermägen der Wiederkäuer. *Biochem. Zeitschr.*, 156:130-137.
- SCHWARZ, C., UND D. G. BIENERT.  
1926. Über die ernährungsphysiologische Bedeutung der Mikroorganismen im Darmtrakt der Pflanzenfresser. I. Mitteilung. Über die Stickstoffverteilung in Coccuminhalt des Pferdes. *Pflügers Arc. f. d. Gesam. Phys. d. Mensch. u. d. Tier*, 213:556-562.
- SHARP, R. G.  
1914. *Diplodinium ccaudatum* with an account of its neuromotor apparatus. *Univ. Calif. Pub. Zool.*, 13:43-122.
- SLOSSON, E. E.  
1928. *Snapshots of Science*. New York.
- STEIN, F.  
1858. Ueber mehrere neue im Pansen der Wiederkäuer lebende Infusionsthier. *Abh. d. Kais. Böhm. Ges. Wiss.*, 10:69-70.  
1859. Charakteristik neuer Infusorien Gattungen. *Lotos*, 9:2-5; 67-70.  
1861. Ueber d. Conjugation d. Infusionsthier u. über d. geschlechtliche Fortpflanzung d. Stentoren. *Sitzber. d. Kais. Böhm. Ges. d. Wiss.* (1861), pp. 62-77.  
1867. *Der Organismus der Infusionsthier*. Leipzig.
- TEN KATE, C. G. B.  
1928. Das Fibrillensystem der Isotrichen. *Arc. f. Prot.*, 62:328-354.
- TRIER, H. J.  
1928. Der Kohlehydratstoffwechsel der Panseninfusorien und die Bedeutung der grünen Pflanzenteile für diese Organismen. *Zeitschr. f. vergl. Phys.*, 4:305-330.
- WEILL, ROBERT.  
1929. Notes Protistologiques Indochinoises. 2. La Présence d'un Infusoire du genre *Isotricha* (*I. caulleryi* n. sp.) chez un Insecte (*Periplaneta americana* Forbes) et sa signification possible. *Arc. Zool. exp. et gen.* 69 (Notes et Revue No. 1):21-26.



- WEISS, D. C. F. H.  
1869. *Specielle Physiologie der Haussäugethiere für Tierärzte und Landwirthe.* Stuttgart.
- WINOGRADOWA-FEDOROWA, T., UND M. P. WINOGRADOFF.  
1929. *Centralbl. für Bakt., etc., 2. Abt., 73:246.*
- WOODCOCK, H. H., AND G. LAPAGE.  
1913. On a remarkable new type of protistan parasite. *Quar. Jour. Micr. Sci., 59:431-457.*
- ZÜRN, F. A.  
1887. *Die Schmarotzer auf und in dem Körper unserer Haussäugetiere. II. Teil. 2. Aufl., 2. Weimar.*

## (B) Other literature cited.

- ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.  
1925. *Official and tentative methods of analysis. 2nd Ed. Washington, D. C.*
- BECHDEL, S. I., H. E. HONEYWELL AND R. A. DUTCHER.  
1928. Synthesis of Vitamin B in the rumen of the cow. *Jour. Biol. Chem., 80:231-238.*
- CLEVELAND, L. R.  
1925. The ability of termites to live perhaps indefinitely on a diet of pure cellulose. *Biol. Bull., 48:289-293.*
- CROSS, C. F., AND E. J. BEVAN.  
1916. *Paper making. 4th Ed. London.*
- EMERY, F. E., AND B. W. KILGORE.  
1892. Digestion experiments. *N. C. Agric. Exp. Sta. Bull. No. 87 d. Tech. Bull. 4.*  
1894. Digestion experiments. *N. C. Agric. Exp. Sta. Bull. 97.*
- FINGERLING, G.  
1909. Weitere Untersuchungen über den Einfluss von Reisstoffen auf die Milchsekretion. *Landw. Vers. Stat., 71:373-414.*  
1911(a). *Ibid.* *Landw. Vers. Stat., 74:163-182.*  
1911(b). Beiträge zur Verwertung von Kalk und Phosphorsaure Verbindung durch den tierischen Organismus. *Landw. Vers. Stat., 75:1-52.*
- FORBES, E. B., AND H. S. GRINDLEY.  
1923. On the formulation of methods of experimentation in animal production. *Bull. Natl. Res. Coun., 6, Part 2, No. 33.*
- HAGEMANN, O.  
1891. Beitrag zur Kenntniss des Eiweissumsatzes im thierischen Organismus. *Landw. Jahrb., 20:261-291.*
- HANSEN, J.  
1922. Fütterungsversuche mit Harnstoff bei Milchkühen. *Landw. Jahrb., 57:141-190.*
- HUNT, C. H., A. R. WINJER, J. A. SCHULZ AND R. C. MILLER.  
1923. The mineral metabolism of the lactating and dry goat. *Am. Jour. Phys., 66:349-362.*
- KENNEDY, CORNELIA.  
1912. A modification of the Sweeney method for crude fiber. *Jour. Ind. Eng. Chem., 4:600*
- MEHTA, M. M.  
1925. The nature of lignin, its physiological significance and its estimation in timbers. *Biochem. Jour., 19:958-978.*

MORGEN, A., C. BEGER AND F. WESTHAUSER.

1909. Weitere Untersuchung über die Verwertung der nicht eiweissartigen Stickstoffverbindungen der Futtermittel sowie der Ammonsälze durch das milchgebende Tier. Landw. Vers. Stat., 71:1-168.

1910. *Ibid.*, 73:286-395.

MÜLLER, M.

1906. Untersuchungen über die bisher beobachtete eiweisssparende Wirkung der Asparagins bei der Ernährung. Pfl. Arc. Ges. Phys., 112:245-291.

QUISUMBING, A.

1921. Comparative study of conditions affecting the determination of reducing sugar by Fehling solution. Jour. Am. Chem. Soc., 43:1503.

SCHALK, A. F., AND R. S. AMADON.

1928. Physiology of the ruminant stomach. N. D. Agric. Exp. Sta. Bull. 216.

SCHNEIDER, B. H., AND H. B. ELLENBERGER.

1927. Apparent digestibility as affected by length of trial and by certain variations in the ration. Vt. Agric. Exp. Sta. Bull. 27.

SNYDER, H.

1893. Digestion experiments. Minn. Agric. Exp. Sta. Bull. 26.

WOODMAN, H. E.

1927. The mechanism of cellulose digestion in the ruminant organism. Jour. Agric. Sci., 17:333-338.

ZUNTZ, N.

1891. Bemerkungen über die Verdauung und den Nährwerth der Cellulose. Pfl. Arc. Ges. Phys., 49:477-483.

1913. Die Beziehung der Mikroorganismen zur Verdauung. Naturwissenschaften, 1 (1):7-11.

## ADDENDUM

Since this paper was accepted for publication, one of us (E. R. B.), has received from Dr. Ernst Mangold, the director of animal physiology in the Landwirtschaftlichen Hochschule in Berlin, his work, embracing 237 pages, on the digestion of ruminants from the *Handbuch der Ernährung und des Stoffwechsels der landwirtschaftlichen Nutztiere*, published in 1929 by J. Springer of Berlin. Twenty-six pages of this work (pp. 156-182) are devoted to a comprehensive discussion of the infusoria of ruminants. The author considers that it is almost proved that the relationship between the infusoria and their host is one of symbiosis. The work carried on under his direction, mentioned in our paper, is extensively reviewed in support of his contentions. Since we shall discuss his findings relating to the infusoria more critically in a later paper, we shall use this opportunity to call attention to this excellent presentation of the subject and to thank Dr. Mangold for the copy of his work.

Percentage composition in feces and excreta

	Pct. H <sub>2</sub> O	Pct. dry matter	Pct. protein	Pct. ether extract	Pct. ash	Pct. N. F. extract	Pct. crude fiber	Pct. hemicellulose calc'd as dextrose	Pct. pentosan	Pct. alpha cellulose	Amounts fed or voided as gms.	Amts. fed or voided as analysed gms.
Hay	6.29	93.71	16.38	1.93	9.77	33.06	32.57	7.83	12.76	19.03	2975	2847
Grain	7.96	92.04	14.81	4.46	4.42	59.24	9.11	11.90	11.31	6.27	1960	1960
Feces	6.15	93.85	13.37	2.46	9.13	29.31	39.58	14.03	16.61	20.14	1813	1807
Urine			3.32								10,772	10,772

## Intake of nutrients

	Feed corrected gms.	Nitrogen gms.	Protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.
Hay	2847	74.56	466.34	54.95	278.15	941.22	927.27	222.92	363.28	541.78	2667.92
Grain	1960	46.45	290.28	87.41	86.63	1161.10	178.56	233.24	222.26	122.89	1803.98
Total per period	3807	121.01	756.62	142.36	364.78	2102.32	1105.83	456.16	585.54	664.67	4471.90
Per day		8.64	54.04	10.17	26.06	150.17	78.99	32.58	41.82	47.48	319.42

## Excretion of nutrients

	Voiled corrected gms.	Nitrogen gms.	N calc'd as protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.
Feces	1807	38.66	241.60	44.45	164.98	529.63	715.21	253.52	300.14	363.93	1695.87
Urine	10,772	57.22	357.63								
Total per period		95.88	599.23	44.45	164.98	529.63	715.21	253.52	300.14	363.93	1695.87
Per day		6.85	42.80	3.18	11.78	37.83	51.09	18.11	21.44	26.00	121.13
Grams digested			515.02	97.91		1572.69	390.62	202.64	285.40	300.74	2776.03



TABLE 3. *Feeds and Excreta of Goat 1 for Period II (14 Days).*  
Percentage composition of feeds and excreta

	Pct. H <sub>2</sub> O	Pct. dry matter	Pct. protein	Pct. ether extract	Pct. ash	Pct. N. F. extract	Pct. crude fiber	Pct. hemicellulose calc'd as dextrose	Pct. pentosan	Pct. alpha cellulose	Amounts fed or voided as gms.	Amts. fed or voided as analyzed gms.
Hay	6.29	93.71	16.38	1.93	9.77	33.06	32.57	7.83	12.76	19.03	3966.67	3796.18
Grain	7.96	92.04	14.81	4.46	4.42	59.24	9.11	11.90	11.31	6.27	1960	1960
Feces	10.83	89.17	12.03	2.36	10.20	26.30	38.28	12.04	15.93	18.78	2215	2191
Urine			4.38								10,392	10,392

Intake of nutrients												
Feed corrected gms.	Nitrogen gms.	Protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.		
Hay	99.49	621.81	73.27	370.89	1255.02	1236.42	297.24	484.39	722.41	3557.40		
Grain	46.44	290.28	87.42	86.63	1161.10	178.56	233.24	221.68	122.89	1803.98		
Total per period	145.93	912.09	160.69	457.52	2416.12	1414.98	530.48	706.07	845.30	5361.38		
Per day	10.42	65.15	11.48	32.68	172.58	101.07	37.89	50.43	60.38	382.96		

Excretion of nutrients												
Voided corrected gms.	Nitrogen gms.	N calc'd as protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.		
Feces	42.17	263.58	51.71	223.48	576.23	838.71	263.80	349.03	411.47	1953.71		
Urine	72.83	455.17										
Total per period	115.00	718.75	51.71	223.48	576.23	838.71	263.80	349.03	411.47	1953.71		
Per day	8.21	51.34	3.69	15.96	41.16	59.91	18.84	24.93	29.39	139.55		
Grams digested		648.51	108.98		1839.89	576.27	266.68	357.04	433.83	3407.67		

Percentage composition of feeds and excreta

	Pct. H <sub>2</sub> O	Pct. dry matter	Pct. protein	Pct. ether extract	Pct. ash	Pct. N. F. extract	Pct. crude fiber	Pct. hemicellulose calc'd as dextrose	Pct. pentosan	Pct. alpha cellulose	Amounts fed or voided as analyzed gms.
Hay	6.29	93.71	16.38	1.93	9.77	33.06	32.57	7.83	12.76	19.03	4555
Grain	5.45	94.55	13.15	4.13	6.45	63.63	7.19	10.60	10.60	4.65	1871
Feces	6.33	93.67	12.92	2.95	10.75	26.85	40.20	11.81	15.45	19.49	2524
Urine			2.42								10,013

Intake of nutrients

	Feed corrected gms.	Nitrogen gms.	Protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.
Hay	4555	119.38	746.11	87.91	455.02	1505.88	1483.56	356.66	581.22	866.82	4268.49
Grain	1871	39.37	246.04	77.27	120.68	1190.52	134.52	198.33	198.33	87.00	1769.03
Total per period		158.75	992.15	165.18	565.70	2696.40	1618.08	554.99	779.55	953.82	6037.52
Per day		11.34	70.87	11.80	40.41	192.60	115.58	39.64	55.68	68.13	431.25

Excretion of nutrients

	Voided corrected gms.	Nitrogen gms.	N calc'd as protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.
Feces	2524	52.18	326.10	74.46	271.33	677.69	1014.65	298.08	389.96	491.93	2364.23
Urine	10,013	38.77	242.31								
Total per period		90.95	568.41	74.46	271.33	677.69	1014.65	298.08	389.96	491.93	2364.23
Per day		6.50	40.60	5.32	19.38	48.41	72.48	21.29	27.85	35.14	168.87
Grams digested			666.05	90.72		2018.71	603.43	256.91	380.59	461.89	3673.29

TABLE 5. *Feeds and Excreta of Goat 2 for Period II (14 Days).*  
Percentage composition of feeds and excreta

	Pct. H <sub>2</sub> O	Pct. dry matter	Pct. protein	Pct. ether extract	Pct. ash	Pet. N. F. extract	Pet. crude fiber	Pet. hemicellulose calc'd as dextrose	Pct. pentosan	Pct. alpha cellulose	Amounts fed or voided as gms.	Amts. fed or voided as analyzed gms.
Hay	6.29	93.71	16.38	1.93	9.77	33.06	32.57	7.83	12.76	19.03	4760	4555
Grain	5.45	94.55	13.15	4.13	6.45	63.63	7.19	10.60	10.60	4.65	2100	1871
Feces	6.82	93.18	12.59	3.16	11.09	25.67	40.67	11.62	15.87	19.39	2460	2460
Urine			2.90								12,363	

Intake of nutrients												
Feed corrected gms.	Nitrogen gms.	Protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.		
Hay 4555	119.38	746.11	87.91	445.02	1505.88	1483.56	356.66	581.22	866.82	4268.49		
Grain 1871	39.37	246.04	77.27	120.68	1190.52	134.52	198.33	198.33	87.00	1769.03		
Total per period	158.75	992.15	165.18	565.70	2696.40	1618.08	554.99	779.55	953.82	6037.52		
Per day	11.34	70.87	11.80	40.41	192.60	115.58	39.64	55.68	68.13	431.25		

Excretion of nutrients												
Voided corrected gms.	Nitrogen gms.	N calc'd as Protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.		
Feces 2460	49.55	309.71	77.74	272.82	631.48	1000.48	285.85	390.40	476.99	2292.23		
Urine 12,363	57.36	358.53										
Total per period	106.91	668.24	77.74	272.82	631.48	1000.48	285.85	390.40	476.99	2292.23		
Per day	7.64	47.73	5.55	19.49	45.11	71.46	20.42	27.89	34.07	163.73		
Grams digested			87.44		2064.92	617.60	269.14	389.15	476.83	3745.29		

TABLE 6. *Needs and Excreta of Goat 3 for Period I (21 days).*  
Percentage composition of feeds and excreta

	Pct. H <sub>2</sub> O	Pct. dry matter	Pct. protein	Pct. ether extract	Pct. ash	Pct. N. F. extract	Pct. crude fiber	Pct. hemicellulose calc'd as dextrose	Pct. pentosan	Pct. alpha cellulose	Amounts fed or voided as gms.	Amts. fed or voided as analyzed gms.
Hay	6.07	93.93	14.79	2.04	9.85	32.98	34.27	7.83	12.65	20.24	8666.7	7999
Grain	7.26	92.74	12.94	4.02	6.42	62.29	7.07	10.55	10.70	4.64	3150	3150
Feces	6.40	93.60	12.13	2.94	11.03	25.37	42.13	11.54	15.22	19.57	4280	4280
Urine			6.55								14,490	14,490

Intake of nutrients												
	Feed corrected gms.	Nitrogen gms.	Protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.	
Hay	7999	189.29	1183.05	163.18	787.90	2638.07	2741.26	626.32	1014.27	1619.00	7513.46	
Grain	3150	65.22	407.61	126.63	202.23	1962.14	222.71	332.33	337.05	146.16	2921.31	
Total per period		254.51	1590.66	289.81	990.13	4600.21	2963.97	958.65	1351.32	1765.16	10,434.77	
Per day		12.12	75.75	13.80	47.15	219.06	141.14	45.65	64.35	84.06	496.89	

Excretion of nutrients												
	Voiled corrected gms.	Nitrogen gms.	N Protein calc'd as gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.	
Feces	4280	83.07	519.16	125.83	472.08	1085.84	1803.16	493.91	651.42	837.60	4006.08	
Urine	14,490	151.85	949.10									
Total per period		234.92	1468.26	125.83	472.08	1085.84	1803.16	493.91	651.42	837.60	4006.08	
Per day		11.19	69.92	5.99	22.48	51.71	85.86	23.52	31.02	39.89	190.77	
Grams digested			1071.50	163.98		3514.37	1160.81	464.74	699.90	927.56	6428.69	



TABLE 7. *Feeds and Excreta of Goat 3 for Period II (21 Days).*

	Pct. H <sub>2</sub> O	Pct. dry matter	Pct. protein	Pct. ether extract	Pct. ash	Pct. N. F. extract	Pct. crude fiber	Pct. hemicellulose calc'd as dextrose	Pct. pentosan	Pct. alpha cellulose	Amounts fed or voided as gms.	Amounts voided as analyzed gms.
Hay	6.07	93.93	14.79	2.04	9.85	32.98	34.27	7.83	12.68	20.24	8666.7	7999
Grain	7.26	92.74	12.94	4.02	6.42	62.29	7.07	10.55	10.70	4.64	3150	3150
Feces	7.03	92.97	11.40	2.69	10.95	26.23	41.70	11.66	15.53	19.34	4334	4334
Urine			7.17								14,610	14,610

## Intake of nutrients

	Feed corrected gms.	Nitrogen gms.	Protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.
Hay	7999	189.29	1183.05	163.18	787.90	2638.07	2741.26	626.32	1014.27	1619.00	7513.46
Grain	3150	65.22	407.61	126.63	202.23	1969.14	222.71	332.33	337.05	146.16	2921.31
Total per period		254.51	1590.66	289.81	990.13	4600.21	2963.97	958.65	1351.32	1765.16	10,434.77
Per day		12.12	75.75	13.80	47.15	219.06	141.14	45.65	64.35	84.06	496.89

## Excretion of nutrients

	Voided corrected gms.	Nitrogen gms.	N calc'd as protein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.
Feces	4334	79.05	494.08	116.58	474.57	1136.81	1807.28	505.34	673.07	838.20	4029.32
Urine	14,610	167.61	1047.54								
Total per period		246.66	1541.62	116.58	474.57	1136.81	1807.28	505.34	673.07	838.20	4029.32
Per day		11.75	73.41	5.55	22.60	54.13	86.06	24.06	32.05	39.91	191.87
Grams digested			1096.58	173.23		3463.40	1156.69	453.31	678.25	926.96	6505.45

TABLE 8. *Feeds and Excreta of Goat 4 for Period I (21 Days).*  
Percentage composition of feeds and excreta

	Pct. H <sub>2</sub> O	Pct. dry matter	Pct. protein	Pct. ether extract	Pct. ash	Pct. N. F. extract	Pct. crude fiber	Pct. hemicellulose calc'd as dextrose	Pct. pentosans	Pct. alpha cellulose	Amounts fed or voided as gms.	Amounts fed or voided as analyzed gms.
Hay	6.29	93.71	16.38	1.93	9.77	33.06	32.57	7.83	12.76	19.03	8925	8541
Grain	5.45	94.55	13.15	4.13	6.45	63.63	7.19	10.60	10.60	4.65	3150	2806
Feces	6.92	93.08	12.05	2.91	10.50	27.44	40.18	11.49	15.08	18.96	4562	4562
Urine			4.19								20,642	20,642

Intake of Nutrients											
Feed cor-rected gms.	Nitro-gen gms.	Pro-tein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.	
Hay 8541	223.84	1399.02	164.84	834.46	2823.65	2781.80	668.76	1089.83	1625.35	8003.77	
Grain 2806	59.04	368.99	115.89	180.99	1785.46	201.75	297.44	297.44	130.48	2653.07	
Total per period	282.88	1768.01	280.73	1015.45	4609.11	2983.55	966.20	1387.27	1755.83	10,656.84	
Per day	13.47	84.19	13.37	48.35	219.48	142.07	46.01	66.06	83.61	507.47	

Excretion of nutrients											
Voided cor-rected gms.	Nitro-gen gms.	N calc'd as Pro-tein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.	
Feces 4562	87.96	549.72	132.75	479.01	1251.81	1833.01	524.17	687.95	864.96	4246.31	
Urine 20,642	138.38	864.90									
Total per period	226.34	1414.62	132.75	479.01	1251.81	1833.01	524.17	687.95	864.96	4246.31	
Per day	10.78	67.36	6.32	22.81	59.61	87.29	24.96	32.76	41.19	202.31	
Grams digested		1218.29	147.98		3357.30	1150.54	442.03	699.32	890.87	6410.53	

TABLE 9. *Feeds and Excreta of Goat 4 for Period II (21 Days).*  
Percentage Composition of feeds and excreta

	Pct. H <sub>2</sub> O	Pct. dry matter	Pct. protein	Pct. ether extract	Pct. ash	Pct. N. F. extract	Pct. crude fiber	Pct. hemicellulose calc'd as dextrose	Pct. pentosan	Pct. alpha cellulose	Amounts fed or voided as gms.	Amis. fed or voided as analyzed gms.
Hay	6.29	93.71	16.38	1.93	9.77	33.06	32.57	7.83	12.76	19.03	8925	8541
Grain	5.45	94.55	13.15	4.13	6.45	63.63	7.19	10.60	10.60	4.65	3150	2806
Feces	10.00	90.00	11.31	2.26	9.42	24.06	42.95	11.55	15.36	19.85	4519	4519
Urine			5.37								16,504	16,504

Intake of nutrients											
Feed corrected gms.	Nitro-gen gms.	Pro-tein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.	
Hay	8541	223.84	1399.02	164.84	834.46	2823.65	2781.80	668.76	1089.83	1625.35	8003.77
Grain	2806	59.04	368.99	115.89	180.99	1785.46	201.75	297.44	297.44	130.48	2653.07
Total per period		282.88	1768.01	280.73	1015.45	4609.11	2983.55	966.20	1387.27	1755.83	10,656.84
Per day		13.47	84.19	13.37	48.35	219.48	142.07	46.01	66.06	83.61	507.47

Excretion of nutrients											
Voided corrected gms.	Nitro-gen gms.	N calc'd as Pro-tein gms.	Ether extract gms.	Ash gms.	N. F. extract gms.	Crude fiber gms.	Hemicellulose calc'd as dextrose gms.	Pentosans gms.	Alpha cellulose gms.	Dry matter gms.	
Feces	4519	81.78	102.12	425.69	1087.27	1940.91	521.94	694.11	897.02	4067.10	
Urine	16,504	141.80									
Total per period		223.58	102.12	425.69	1087.27	1940.91	521.94	694.11	897.02	4067.10	
Per day		10.65	4.86	20.27	51.77	92.42	24.85	33.05	42.72	193.67	
Grams digested			178.61		3521.84	1042.64	444.26	693.16	858.81	6589.74	



Table 10. *Summary.*

Presence or absence of infusoria	Goat No.	Period No.	Coefficients of Digestibility								Excretion of Nitrogen			Nutri- tive ratio of rations	Av. wt. of goat during period lbs.	Net gain or loss during period lbs.	Length of period days
			Protein	Ether extract	N. F. extract	Crude fiber	Hemi- cellulose	Pento- san	Alpha cellulose	Total dry matter	Pct. of excreted N. voided in feces	Pct. of excreted N. voided in urine	Daily N. balance = gms. of N. stored daily				
Absent		I	68.07	68.78	74.81	35.32	44.42	48.74	45.25	62.08	40.32	59.68	+1.79	1:4.2	37.00	+3.0	14
	1																
Present		II	71.10	67.82	76.15	40.73	50.27	50.57	51.32	63.56	36.67	63.33	+2.21	1:4.1	38.00	0.0	14
Increase or decrease with infusoria present			+3.03	-3.99	+1.34	+5.41	+5.85	+1.83	+6.07	+1.48			+0.42				
Absent		I	67.13	54.92	74.87	37.29	46.29	49.98	48.43	60.84	57.37	42.63	+4.84	1:4.2	34.50	+0.75	14
	2																
Present		II	68.78	52.94	76.58	38.17	48.49	49.92	49.99	62.03	46.35	53.65	+3.70	1:4.2	36.25	-2.00	14
Increase or decrease with infusoria present			+1.65	-1.98	+1.71	+0.88	+2.20	-0.06	+1.56	+1.19			-1.14				
Absent		I	67.36	56.58	76.40	39.16	48.48	51.79	52.55	61.61	35.36	64.64	+0.93	1:4.7	59.25	-2.25	21
	3																
Present		II	68.94	59.77	75.29	39.03	47.29	50.19	52.51	61.39	32.05	67.95	+0.37	1:4.6	58.00	+2.00	21
Increase or decrease with infusoria present			+1.58	+3.19	-1.11	-0.13	-1.19	-1.60	-0.04	-0.22			-0.56				
Absent		I	68.91	52.71	72.84	38.56	45.75	50.41	50.74	60.15	38.86	61.14	+2.69	1:4.0	86.00	+1.00	21
	4																
Present		II	71.09	63.62	76.41	34.95	45.98	49.97	48.91	61.84	36.58	63.42	+2.82	1:4.0	83.00	-1.00	21
Increase or decrease with infusoria present			+2.18	+10.91	+3.57	-3.61	+0.23	-0.44	-1.83	+1.69			+0.13				
Average figures with infusoria absent			67.87	58.25	74.73	37.58	46.23	50.23	49.24	61.17							
Average figures with infusoria present			69.98	61.04	76.11	38.22	48.01	50.16	50.68	62.21							
Average of all figures			68.92	59.64	75.42	37.90	47.12	50.20	49.96	61.69							
Median figure with infusoria absent			68.02	60.75	74.62	37.24	46.45	50.27	48.90	61.12							
Median figure with infusoria present			69.94	60.38	75.94	37.84	49.13	50.25	50.71	62.48							
Median of all figures			69.12	60.75	74.71	37.84	47.35	50.27	48.90	61.86							





## THE PIGEON FLY AND PIGEON MALARIA IN IOWA

C. J. DRAKE AND R. M. JONES\*

*From the Department of Zoology, Iowa State College.*

Accepted for publication December 1, 1929.

During the past summer, 1929, pigeon fanciers and commercial squab raisers in Iowa and several southern states have been much concerned by the presence of a parasitic fly which greatly worried and annoyed their birds. As the summer advanced, the flies kept increasing in numbers and the pigeons became increasingly disturbed and restless. The colder weather this fall, however, has checked reproduction and the flies have been slowly decreasing. Although at the present time (December) the flies are fairly common on the pigeons, apparently no purparia have been deposited in the nests and cotes since the latter part of October. Puparia, collected during September and kept in containers in the pigeon houses, are still viable and apparently normal. Further experiments are being carried on to determine whether or not the flies and puparia will survive the winter weather of Iowa, and if the flies survive, whether or not they will continue to breed and deposit puparia during the winter.

The pigeon fly, *Pseudolynchia maura* (Bigot), was first reported from Ames, Iowa, by Knab in 1916. He also recorded the insect from Key West, Florida, and Savannah, Georgia. The latter seems to be the earliest published record of its occurrence in North America, the specimens being taken on pigeons at Savannah, Sept. 28, 1896, by W. Duncan. Bequaert (1925) and Bishopp (1929) have published notes and records of the pigeon fly from Florida, Georgia, South Carolina, Alabama, Louisiana, Texas, Arkansas, southern California and a number of foreign countries. The numerous records from southern Europe, Africa, Asia Minor, Philippine Islands, India, Hawaii, West Indies, South America, North America, and various other regions illustrate how the relationship of a host and its close parasite, both of them alternate hosts to the organisms of a blood disease, may be the main factor in the spread of such a disease into country after country. In this case the importation of pigeons infested with pigeon flies and both bird and fly infected with malarial organism has carried the disease into North America. The Iowa infestation resulted from such shipments from Florida. After studying an extensive series of the fly from many parts of the world, Bequaert was inclined to believe that *P. maura* was originally an old world insect, which was introduced by man into the Americas, together with the domestic pigeon.

---

\*The writers are greatly indebted to Dr. E. R. Becker, Iowa State College, for studying the blood of the pigeons and identifying the pigeon malaria parasite; also to Dr. J. M. Aldrich of the U. S. National Museum for checking up the determination of the hippoboscoid fly; and to Mr. A. R. Janson of New York City for making the illustrations. Thanks are also due to Mr. Charles Foy, Clinton, Iowa, for assistance in the control experiments and for the donation of pigeons for experimental purposes.

The pigeon fly (Pl. I, *a*) is a strange-looking, highly specialized parasite belonging to the dipterous family Hippoboscidae. Its body is strongly flattened, leathery, brownish in color and provided with large legs and long, slender wings. The powerful legs and other special adaptations enable the parasite to crawl rapidly about on the body or through the feathers of its host, or to take sudden flight when the bird is handled. The tarsal claws (Pl. I, *c*) are also large, strongly bent and provided with long teeth, thus enabling the fly to cling tightly to the feathers. As a result it is quite difficult for the pigeon itself to dislodge the fly. On its host or elsewhere, the fly is always on the alert and capable of moving quickly, generally running sideways in a somewhat crab-like manner. This mode of locomotion is quite similar to that of other species of hippoboscids found upon a number of our native species of wild birds.

*Pseudolynchia maura*, like other members of the family, is a blood-sucker and not capable of living off of its host for any considerable period of time. Its feeding habits and movements greatly annoy and provoke much worry and irritation among the squabs, fledglings and older birds. Heavily infested pigeons frequently spend no small part of their time fighting the flies by pecking and thrusting their beaks among their feathers. This pecking causes injury and deterioration in the general appearance and quality of the feathers, both of which are of commercial importance to raisers of fancy and breeding birds. In addition to the injury and annoyance caused directly by its feeding and irritating movements on the bird, the fly also serves as an intermediate host and is known to transmit two disease-producing protozoa of the common pigeon (*Columba livia*). An outbreak of one of these diseases, pigeon malaria, was found in Iowa during the past summer.

An examination of preparations of both fresh and dried stained films of blood from pigeons infested by *Pseudolynchia maura* (Bigot) showed the presence of a haemoproteid parasite, *Haemoproteus columbae* Celli and Sanfelice (1891), in large numbers. This halteridium is the causative organism of a disease of the pigeon, called pigeon malaria, which has been known in India, Africa, Europe (southern part), and South America for many years. Doctors Edw. and Et. Sargent first convicted the pigeon fly of carrying and transmitting pigeon malaria in 1906. Since then, a considerable amount of work has been done upon the ethology and morphology of *Haemoproteus columbae* (Pl. II) in the pigeon (asexual cycle) and in the pigeon fly (sexual cycle) by Aragao, Negri, Acton and Knowles, Adie, Gonder, Wenyon, and others, but not much attention has been given to the biology and control of the intermediate host, the pigeon fly, which acts as a biological carrier of the disease from pigeon to pigeon. Although the other species of a protozoan, *Trypanasoma hunnai* Pitagula (1904), was not found in Iowa, further examination of fresh and dried stained smears of blood from infested pigeons in the southern states may also show the presence of this parasite in the United States. Aragao (1927) has reported the presence of this trypanosome both in the pigeon fly and pigeons in Brazil, South America.

In collecting several hundred pigeon flies upon unfledged and older pigeons, many of the flies settled upon the exposed skin and clothing of the writers, but in no instance did they bite. When the birds are handled,

the flies taking wing do not, as a rule, wander very far away, and before returning and concealing themselves on their hosts, they generally perch for a few moments upon nearby objects. The pigeon fly is practically a permanent parasite and spends almost all of its existence as a fly upon its host. It was difficult to determine whether the disturbance of the birds was due largely to the feeding habits of the flies, or to the tickling and mechanical irritation resulting from the crawling and other activities of the flies upon the pigeons. Nevertheless, their mode of life is exceedingly irritating and causes marked restlessness and worry to the birds.

Observations indicate that the pigeon fly seldom leaves or flies away from its host under normal conditions, except for certain special purposes as when forced to establish new quarters due to the death of its host or when a parturient female seeks a favorable place to deposit a mature larva. Ordinarily, the flies make little use of their wings. If, however, the birds are caught and the feathers parted and ruffled to locate the flies, many of the disturbed individuals make very short, quick flights and frequently settle upon the nest boxes, walls, or other objects. On the other hand, the pecking at the flies or the ruffling of the feathers by the bird itself rarely results in the flies taking flight.

Within a few minutes after being captured and placed in glass vials a number of female flies gave birth, one at a time, to full-grown larvae, which began to transform into the resting stage immediately after deposition. At the time of extrusion the mature larva is enclosed in an obovoid capsule and pupal case, it is incapable of locomotion, and is whitish in color with a black cap-like structure at the caudal end. As the transformation continues, the puparium (Pl. I, *d*) successively turns from white through yellow, orange, light to dark brown, and in the course of two to three hours becomes strongly indurated, somewhat shiny and jet black in color.

A number of the nests in the infested pigeon houses were equipped with false sliding bottoms to facilitate cleaning. On the floors under the false bottoms of 60 nests, 255 puparia were collected in about two hours. Sometimes, as many as 10 puparia were found beneath a single false bottom. The puparia were much more difficult to find in the nests without the false bottoms. In the latter they were more or less concealed in the dust, chaff, stems, and faecal matter. Several infested pigeons for experimental purposes were confined in small boxes having the water and food pans attached to one side and almost resting on the floor. In these cages a large percentage of the puparia were deposited on the floor beneath the pans. These and other records indicate that the female flies frequently consign the puparia to a less or more protected situation for the developmental period.

Several puparia, deposited in glass vials during the latter part of September, required 35 days for the transformation of the pupal stage. In Africa, Bedford (1924) states that the pupal stage lasts from 23 to 28 days. In the southern part of the United States, Bishopp (1929) reports the resting stage to last for 20 to 30 days. No records of this stage have been secured for the summer season in Iowa. Soon after a fly emerges, it is ready for a meal of the pigeon's blood, which is absolutely essential for the life of the fly.



The adult fly remains almost constantly upon the pigeon and is not known to feed upon other domesticated birds and fowls or wild birds in America. Its mouthparts are highly developed for sucking blood and feeding takes place only in the adult stage.

Experiments for the control of the pigeon fly were conducted at Clinton and Ames, Iowa, during the latter part of the past summer, 1929. These consisted of cleaning up measures to destroy the puparia and some preliminary tests with certain insecticides used in the form of dusts, dips, sprays, and paints. As the resting stage is passed largely in the nests and lasts from 20 to 35 days, the infestation may be greatly reduced by carefully cleaning out and destroying the nesting materials as frequently as possible, preferably every 20 days. Bishopp also found that a light application of a commercial household fly spray (kerosene-pyrethrum extract) upon the nests and birds destroyed many of the flies. These sprays should be applied very lightly, with a good atomizer.

Pyrethrum and derris powders were used both as dusts and as dips. In the dust form the powders were sifted quite liberally among the feathers of the birds. The pyrethrum dip consisted of five ounces of the powder added to five gallons of rain or soft water in which two and a half ounces of laundry soap had been dissolved. The derris dip was prepared in a similar manner.

Nicotine sulfate was used both as a nest paint and as a dip. The walls of a few nests were painted with nicotine sulfate (40%) in the evening, and the following morning the pigeons were caught and examined for pigeon flies. In addition, several pairs of birds were confined for one night in small boxes having the walls freshly painted with nicotine sulfate and the open side closed with a wire screen for ventilation. After a period of from 12 to 16 hours a number of flies were found dead in the nests and upon the floor of the cages, but it was not difficult to find living flies on the birds. The nicotine fumes seemed to have some deleterious effect upon the pigeons, especially the young birds in the nest, but no birds were seriously injured or killed in the experiments. In the form of a dip, nicotine sulfate was used at the rate of one ounce in six gallons of soft water containing four ounces of dissolved laundry soap. The results secured with the nicotine dip were quite satisfactory.

Pyrethrin-soap as prepared by Van Leeuwen (1926) and modified by Van Leeuwen and Van Menden (1927) of the U. S. Bureau of Entomology, proved satisfactory as a dip for the control of pigeon flies. This formula consists of 86.5 ounces of sodium oleate, 9.5 ounces of the alcoholic extract of pyrethrum flowers, and 6 fluid ounces of sodium silicate. This material is manufactured and sold commercially under the trade name "Red Arrow." It is non-poisonous and was used as a dip at the rate of one ounce of the commercial concentrate in six gallons of soft water. After carefully cleaning out and disposing of all manure, dirt, and nesting materials so as to insure the death of the puparia, the walls, floors, nests and outdoor yards were thoroughly sprayed with waste crank case oil. The cleaning and spraying operations, coupled with the dipping of all pigeons in the pyrethrin-soap dip, completely eradicated the flies on one of the larger pigeon farms of the state. On this farm over two thousand pigeons were dipped on warm, sunny days during the first part of October without the loss, or even

apparent injury, of a single bird. In some of the preliminary tests a number of pairs of fancy birds were dipped in pyrethrin-soap solution and the following day the pigeons were placed in shipping crates and sent to other bird fanciers. No apparent injury to the birds was observed by the pigeon fanciers.

## LITERATURE CITED

ADIE, HELEN.

1924. The Sporogony of *Haemoproteus columbae*. Bull. Soc. Patholog. exot. 17:605-613, 2 pls. and 6 figs.

ARAGAO, HENRIQUE DE BEAUREPAIRE.

1908. Über den Entwicklungsgang und die Übertragung von *Haemoproteus columbae*. Arch. f. Protistenk., 12:154-166. Pls. XI-XIII.

---

1927. Evolution de l'*Haemoproteus columbae* et du *Trypanosoma hunnai* dans la *Lynchia maura* Bigot. C. R. Soc. Biol., 11-827-829, 3 figs.

AUSTEN, ERNEST EDWARD.

1909. The African blood-sucking flies. 221 pp. Pls. I-XIII (Hippoboscidae, 7:163-178).

BEDFORD, G. A. H.

1924. The external parasites of poultry, with measures for their control. Jour. Union S. A., Dept. Agric., 123-140 (The Pigeon Fly, 140).

BEQUAERT, J.

1925. Notes on Hippoboscidae. Psyche., 32:265-277.

BISHOPP, F. C.

1929. The pigeon fly—an important pest in this country. Amer. Pigeon Jour., 18:419-420, 1 fig.

FERRIS, G. F.

1925. Third report upon Diptera pupipara from the Philippine Islands. Phil. Jour. Sci., 27:413-421, 5 figs. (*Lynchia maura*, figs. 2 and 3).

KNAB, FREDERICK.

1916. Four European Diptera establishes in North America. Insector Inscitiae Menstr., 4:1-4.

VAN LEEUWEN, E. R.

1926. A contact spray for the control of the Japanese beetle (*Popilia japonica* Newm.). Jour. Econ. Ent., 19:786-790.

VAN LEEUWEN, E. R., AND P. A. VAN DER MEULEN.

1927. Further information on a contact spray for the control of the Japanese beetle (*Popillia japonica* Newm.). Jour. Econ. Ent., 20:603-607.

WENYON, C. M.

1926. Protozoology, 2:888-897 (*Haemoproteus* of birds), (figures of *Lynchia maura* and *Haemoproteus columbae*).

## PLATE I.

Pigeon Fly, *Pseudolynchia maura* (Bigot); *a*, dorsal aspect of male; *b*, fringe at postero-lateral angle of scutellum; *c*, posterior tarsus; *d*, puparium of pigeon fly.

PLATE I.

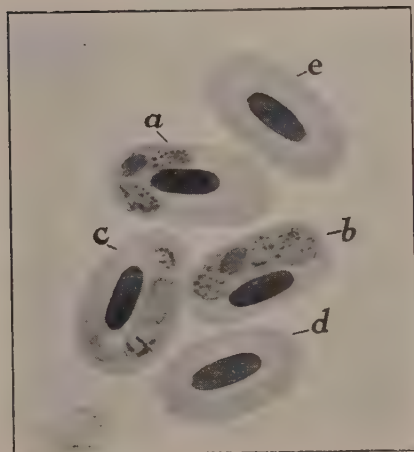




## PLATE II.

*Haemoproteus columbae* in blood of pigeon; *a* and *b*, female gametocyte; *c*, male gametocyte; *d* and *e*, normal gametocytes.

PLATE II.





# STUDIES OF CROWNGALL, OVERGROWTHS AND HAIRYROOT ON APPLE NURSERY STOCK

J. H. MUNCIE AND ROSS F. SUIT\*

*From the Department of Botany, Iowa State College.*

Accepted for publication December 10, 1929.

Following the discovery of *Pseudomonas tumefaciens* Sm. and Town. as the cause of crowngall of Paris daisy (16) and other plants, various overgrowths on apple stock, both above and below ground, not known to be due to another organism have been classed as manifestations of this disease. Likewise, the condition in apple and quince trees in which an abundance of fine fibrous or fleshy roots arise singly or in clusters from the base of the trunk, the crown, or roots, has been known as hairyroot since the original description by Stewart, Rolfs and Hall (18).

Subsequently, Smith and his co-workers (17) established the relation of *Ps. tumefaciens* to one form of hairyroot. In specimens which they studied, clusters of small fibrous and fleshy roots arose from the main root in the absence of a typical crowngall. There were, however, small enlargements at the bases of the root clusters. In six of the nine trials, isolations from the flattened tumorous swelling from which the roots arose yielded the crowngall organism. Subsequent inoculations into young apple and quince trees and sugar beets resulted in the production of both galls and hairyroot.

More recent investigations by Riker and Keitt (10) (11) (12) and the senior writer (7) have shown that there are similar overgrowths from which no causal organism has been isolated as well as similar abnormalities due to different causes. It seemed advisable, therefore, to extend the present investigations of crowngall, overgrowths, and various types of hairyroot to determine their occurrence and prevalence in the nurseries and to study their relationship to *Ps. tumefaciens*.

## THE PREVALENCE OF CROWNGALL, OVERGROWTHS AND HAIRYROOT ON APPLE NURSERY STOCK

Estimates by nurserymen, together with field observations made at various nurseries when the apple trees were dug, have shown that a relatively large percentage of the trees are discarded annually because of crowngalls, overgrowths and hairyroot. The losses due to various malformations may vary considerably, depending upon the variety, soil and seasonal conditions. In certain varieties, during unfavorable growing seasons, 85 per cent of the trees may be discarded. Losses of 10 to 35 per cent are

---

\*The writers wish to acknowledge their indebtedness to Dr. I. E. Melhus, under whose direction this work was done, for helpful criticism and suggestions received throughout the course of these investigations.

These studies have been carried out in connection with the crowngall project in which the Crop Protection Institute of the National Research Council, University of Wisconsin, United States Department of Agriculture, Office of Mycology and Disease Survey, and Iowa State College are cooperating.



not uncommon for many varieties. Piece-root grafted trees suffer more severe losses due to these causes than those propagated by budding.

In order to determine the annual loss on apple stock, field examinations were made in 1925, 1926 and 1927 of several thousands of budded and grafted apple trees when they were being dug at various nurseries in several midwestern, eastern and southern states. Thirty-two nurseries in 13 states were visited and critical examinations made of 55,668 grafted and 30,632 budded apple trees. The survey included representative apple growing nurseries in the states of Iowa, Illinois, Indiana, Nebraska, Kansas, Missouri, Oklahoma, Michigan, New York, Alabama, Tennessee, Kentucky and Virginia.

During the survey of 1925, more attention was given to the general occurrence of crown gall as compared with that of overgrowths and hairy-root. In some cases, apple trees which had been discarded because of overgrowths were carefully examined and in others, counts were made at the time the trees were dug to determine the relative percentage affected with the true crown gall and overgrowths.

Again, in 1926 and 1927, representative nurseries in the Missouri and Mississippi valleys, and in the eastern and southern United States were visited, and examinations made of several varieties of apple stock as they were dug. The varieties included those which usually showed a high as well as a low percentage of trees discarded because of overgrowths. In this way, the average loss to the crop as a whole could be determined with some degree of accuracy.

Attempts were made to examine equal numbers of grafted and budded apple trees of the same varieties at each nursery in order to determine the relative percentage of overgrowths and true crown gall. This was impossible in actual practice, but sufficiently large numbers of trees of several varieties were examined to show the relative prevalence of crown gall, overgrowths and hairyroot upon both budded and grafted nursery apple trees. The data collected in the surveys of 1925, 1926 and 1927 are presented in summarized form in table 1.

TABLE 1. *Relative Prevalence of Crown gall, Wound Overgrowths and Hairyroot on Budded and Grafted Nursery Apple Trees.*

Year	States	Nurseries visited	Method of propagation	Number varieties	Number trees	Percentage trees with		
						Crown-gall	Over-growths	Hairy-root
1925	3	3	grafted	10	6809	1.26	16.8	2.7
	2	4	budded	6	9668	1.02	6.7	3.4
1926	5	18	grafted	35	20756	0.35	36.1	1.57
	7	10	budded	27	16602	0.29	7.6	0.69
1927	5	10	grafted	49	28103	0.36	45.4	0.65
	2	3	budded	13	4362	0.04	6.53	0.84

The data in table 1 show that for the 55,668 piece-root grafted trees examined, the percentage of overgrowths varied from 16.8 to 45.4. In the

30,632 budded trees, those discarded because of overgrowths varied from 6.53 to 7.6. The difference in the percentage of overgrowths on piece-root grafted and budded stock can be explained readily by the difference in the method of propagation. The formation of overgrowths at the union of piece-root grafted trees may be accounted for largely on the basis of wounding, as the result of grafting, and the imperfect fitting of scion and stock as shown by Riker and Keitt (10), Melhus (6), and Muncie (7). Overgrowths above the union on grafted and on the stocks of budded trees frequently develop as the result of cultivation wounds.

It is significant, as shown in table 1, that only a small percentage of all the trees examined, either budded or grafted, show overgrowths, which by means of field examination would be classed as bacterial crowngalls, and that the occurrence of crowngalls upon grafted and budded stock is in relatively the same proportion. Of the grafted 0.35 to 1.26 per cent and of the budded trees 0.04 to 1.02 per cent showed true crowngalls. These data were based upon field diagnosis, in which overgrowths without roots or root primordia, lacking bark or a cork covering, of a creamy white color, and which oxidized rapidly upon exposing the cut surface to the air, were considered to be true crowngalls. The presence of such a gall was regarded as evidence of primary infection by *Ps. tumefaciens*. During the 1926 survey, 42 specimens collected by field men as suspected crowngalls were sent to the laboratory for isolation trials. Similarly, during the 1927 survey, isolations were made from 43 specimens suspected of being true crowngall. Of the 42 specimens employed in isolation and inoculation trials, the pathogen was recovered in four cases; from the other lot of 43 specimens, *Ps. tumefaciens* was recovered in 24 cases. The results of isolation studies upon suspected crowngalls show the difficulty of accurate diagnosis of this disease under field conditions, and also the relatively small percentage of true crowngall present in the nursery apple blocks.

As shown in table 1, only a small percentage of either budded or grafted nursery apple trees show the hairyroot condition in the absence of overgrowths. The total percentage of hairyroot is shown in this table. The data collected, however, show for each year, approximately twice the amount of the fibrous form as compared with the fleshy form of hairyroot. No distinction was made in collecting the data as to whether the hairyroot condition was of the burr-knot type or of the fibrous or fleshy form. Observations showed, however, that the burr-knot type was less commonly found. Of the knotted trees, approximately 80 per cent were typical of the hairy-knot condition, in which an abundance of fibrous roots arose from the overgrowth. In some cases, fleshy roots were present also, either alone or associated with the fibrous roots. As will be shown later, isolations to determine the presence of *Ps. tumefaciens* were made from numbers of apple trees and seedlings showing the different types of hairyroot.

It has been shown previously (6), (7), (10) that the improper fitting of the elements of the graft have a direct bearing upon the production of knotted trees, and that overgrowths may be induced following mechanical injury of the scion. Further data were obtained from the trees examined during 1925, 1926 and 1927, showing the location of the overgrowths and their probable relation to the process of grafting and cultivation injuries. These data, summarized in table 2, show that an average of 61.3 per cent

of the overgrowths occurring on the piece-root grafted trees were formed on the tip of the scion lip. In previous experiments by Riker and Keitt (10) and the writer (7) it has been shown that overgrowths develop readily from the tip of the scion lip when it is allowed to project beyond the cut surface of the stock of the graft. Likewise, the improper matching of the cambium of stock and scion leads to the development of overgrowths at this point. The field data show 14.1 per cent of the knotted trees with overgrowths at some point along the side of the union. Only 5.3 per cent of the knotted trees bore overgrowths at the tip of the stock lip. If the stock lip does not completely cover the cut surface of the scion, overgrowths may develop from the scion at this point. This condition is commonly found on varieties such as Wealthy and Winter Banana, when grown in the southern nurseries under conditions suitable for rapid growth. In the northern nurseries, where growth is less rapid, the cut surface of the scion not covered by the stock lip may produce a smooth normal callus.

TABLE 2. *Percentage of Knotted Piece-root Grafted Apple Trees and Location of the Overgrowths as Determined by Field Examinations in 1925, 1926 and 1927.*

Year	States	Nurs- eries	Varie- ties	Trees exam- ined	Knotted trees		Location of knots and per cent				
					Per cent	Num- ber	On scion tip	At side of union	On stock tip	Above union	Below union
1925	3	3	10	6809	16.8	1144	75.8	8.9	5.12	4.5	5.3
1926	5	18	57	20756	36.1	7497	43.8	22.5	9.4	13.0	11.2
1927	5	10	49	28103	45.4	12838	70.3	10.4	2.9	11.6	4.6
Total and average				55668	38.5	21479	61.3	14.1	5.3	11.7	6.9

Although these data indicate that the misfitting of the elements of the graft may be responsible for a majority of the overgrowths, there is a certain percentage of these which apparently is induced as a result of wounding in cultivation. For the most part, these are confined to the underground portion of the scion above the union. Thus, it is shown that an average of 11.7 per cent of all the knotted trees examined showed callus knots upon the lower part of the scion. This loss is to a large extent avoidable. Below the union, overgrowths frequently develop at some point on the stock as a result of mechanical injury after planting, or upon the basal end of the root where it has been severed in making the piece-root graft. No accurate records were made of the occurrence of overgrowths at various places upon the stock, but observations indicate that a majority of them arose from the basal end of the root or stock. On 6.9 per cent of the trees with overgrowths, the knot occurred at some point upon the stock below the union.

#### ISOLATION STUDIES UPON OVERGROWTHS AND HAIRYROOT

Although field diagnosis of the overgrowths on budded and piece-root grafted apple trees indicated that less than one per cent was caused by crown gall infection, isolations were made from a large number of specimens to supplement these observations. Similarly, apple trees and one-



year-old seedlings showing different types of the hairyroot condition were also employed in isolation studies.

#### METHODS

In the isolation studies the following method was employed. The portion bearing the overgrowth was severed from the tree, scrubbed thoroughly in running tap water and dried with clean blotting paper. The overgrowth was then scraped with a scalpel to remove, as far as possible, particles of soil which might have lodged in the crevices formed by the convolutions of the surface.

The surface of the overgrowth was covered with 95 per cent alcohol, and flamed. Small pieces of tissue, approximately one centimeter square and 3 to 5 millimeters in thickness, were removed with a flamed scalpel and forceps and placed in a sterile petri dish. About two cubic centimeters of sterile water were added to the petri dish and the tissue was then finely macerated with a flamed scalpel. The suspensions of macerated tissue after standing for one-half hour to one hour were ready for making the poured plates. The suspension was placed in 25 cubic centimeters of bile agar, using Patel's (8) formula, and with this three plates were poured. The plates were incubated at room temperature and notes taken upon the character of the bacterial colonies which developed. Control plates were made, using suspensions of macerated tissue from a true crown gall known to be infectious. Colonies having the appearance of *Ps. tumefaciens* were streaked upon potato dextrose agar. Unless otherwise stated, inoculations were made into healthy tomato plants from (1) the suspensions of macerated tissue of the overgrowths, (2) from the suspensions made by flooding plates containing colonies resembling the crown gall bacteria and (3) from the streak cultures of the organisms having the appearance of *Ps. tumefaciens*.

The inoculated plants were incubated in a moist chamber for two to four days without watering, and then placed upon a greenhouse bench for observation. No inoculated plants were discarded until at least 30 days after inoculation.

#### ISOLATIONS FROM PIECE-ROOT GRAFTED TREES WITH HAIRY-KNOT

In a previous publication (7) the results of isolations from 155 young nursery apple trees with hairy roots on the overgrowths at the union have been given. In these trials, the strain of the crown gall organism producing galls on young tomato plants was recovered from 16 of the trees so affected. During the fall of 1926, apple trees showing overgrowths at the union with fibrous hairy roots (hairy knots) were collected at various nurseries at the time of digging. This material, including trees of the varieties Wolf River, Gano, and Yellow Transparent, from four nurseries in Kansas and Tennessee, was employed in isolation studies in 1927. The trees so employed were typical of those discarded by the nurserymen as affected with hairy-knot (woolly knot as described by Hedgecock (4)).

The first lot of specimens employed in the isolation trials consisted of 47 trees of the variety Wolf River collected at a Kansas nursery (Plate IA). The overgrowths were conspicuous, usually half surrounding the tree at the union. The surface of the malformation was rather finely wrinkled with a bark layer and covered with small, fibrous, and fleshy roots. The interior of the overgrowth, in a majority of cases, was hard and woody be-



low a depth of about five millimeters. In 20 specimens the overgrowth originated at the tip of the scion lip, in 16 along the sides of the union, in 10 from a wound on the scion above the union, and in one case from the tip of the stock lip. The size of the overgrowths varied somewhat, but the average was one and one-half inches long, one and one-half inches wide, with an elevation of three-fourths inch.

Colonies resembling *Ps. tumefaciens* were found in plates from 31 of the trees. In plates from the other 16 trees, white, circular bacterial colonies not resembling *Ps. tumefaciens* appeared in plates from 13, and from three, the plates were sterile.

Inoculations into tomato plants were made from suspected *Ps. tumefaciens* colonies from each of the 31 trees. The plates were then flooded with sterile water and the suspension also inoculated into young tomato plants. Inoculations were also made from the suspension of macerated tissue after the plates were poured. In all cases the results were negative.

Isolations were next made from a lot of 14 Gano apple trees collected from another Kansas nursery. All of these specimens showed hairy-knots typical of those discarded by the nurserymen. In one specimen, the lateral root from the union terminated in a mass of fibrous roots commonly referred to as "broom root." The location of the overgrowths was as follows: seven at the side of the union, four at the tip of the scion lip, two on the scion above the union and one on the tip of the stock lip. The overgrowths were smaller in size than those of the previous lot of Wolf River trees, averaging three-fourths inch long, three-fourths inch wide with an elevation of one-half inch. Five of the overgrowths bore fleshy as well as fibrous roots. Plate cultures from three trees showed colonies resembling *Ps. tumefaciens*, from one tree the plates were sterile and from the remaining 10 trees white, dense, circular colonies unlike the crown gall organism were obtained. The crown gall organism was not recovered from any of the specimens, as determined by inoculation into tomato plants.

A third lot of material consisting of 39 one-year-old Yellow Transparent apple trees was received from a Tennessee nursery. These were selected by one of the state nursery inspectors as typical of hairy-knot on grafted trees. In all cases the hairy roots were fibrous, but on one tree the tips of the roots were slightly fleshy. The overgrowths were small, averaging three-fourths inch long, three-fourths inch wide and with an elevation of one-half inch. Twenty of the trees showed overgrowths at the tip of the scion lip; 12 at the side of the union; three at the tip of the stock lip; one on the scion above the union, and three on the stock below the union.

From 13 trees, organisms were obtained which closely resembled *Ps. tumefaciens* in plate culture; poured plates from five of the specimens were sterile. Plates from the remaining 21 trees showed bacterial colonies, but these did not resemble the crown gall organism. Inoculations were made into young tomato plants from sub-cultures of the crown gall-like organisms, and with suspensions from the plates showing bacterial colonies. Likewise inoculations were made also with the suspensions of macerated tissue. In no case was the crown gall organism recovered.

On November 10, a lot of 22 piece-root grafted one-year-old apple trees of the variety Northwestern Greening was collected at the time of digging from a Kansas nursery. The overgrowths of these trees averaged one and

one-half inches long, one inch wide, with an elevation of three-fourths inch. In all cases, the surface of the knot was rough and wrinkled, firm, but soft in texture to a depth of about 5 millimeters. The overgrowth in each case was covered with a bark layer and bore an abundance of fibrous roots. Of the overgrowths nine occurred upon the scion above the union, seven along the side of the union, three at the tip of the scion lip, and one below the union on the stock. Isolations from these specimens failed to yield the crowngall bacteria.

Likewise, similar isolations were made from a lot of 15 Golden Winesap and 10 Wealthy two-year "cut-back" apple trees collected at an Iowa nursery. These trees bore overgrowths typical of those discarded by the nurserymen as hairy knot. The overgrowths were wrinkled, with a definite bark layer, soft in texture to a depth of about five mm. and with an abundance of fibrous roots. The crowngall pathogen was not recovered from any of these specimens.

A third lot of specimens consisting of seven Yellow Transparent and 15 Wealthy apple trees collected at a Nebraska and an Iowa nursery, respectively, was employed in isolation trials. These trees were grown as two-year "cut-backs" from piece-root grafts. The overgrowths were similar to those previously mentioned. A majority, or 13, of the knots occurred at the side of the union—six at the tip of the scion lip, two at the tip of the stock lip, and one on the scion above the union. The overgrowths varied somewhat in size, but averaged two and three-fourths inches in length, one and one-fourth inches in width, with an elevation of three-fourths inch. In most cases, the overgrowth involved three-fourths the circumference of the union; in a few cases, the union was completely encircled. All the overgrowths bore fibrous roots typical of the hairy-knot condition. Isolations from these specimens also failed to yield *Ps. tumefaciens*.

Control isolations were made from two crowngalls induced by artificial inoculation on young apple trees and from a gall on Zumbra plum produced by natural infection in the nursery. The crowngall bacteria were recovered in abundance from the control plates.

A fourth lot of material employed in isolation trials was collected at another Tennessee nursery. The lot consisted of 10 one-year-old piece-root grafted Yellow Transparent apple trees. Nine of these trees bore overgrowths averaging one and one-half inches long, one inch wide, with an elevation of three-fourths inch, but the tenth tree showed only a slightly swollen union. The overgrowths were located as follows: five at the tip of the scion lip, three along the side of the union, and one at the tip of the stock lip. These overgrowths were typical of those described as hairy-knots; with woody interior; finely wrinkled surface, covered with a bark layer; and bearing an abundance of fine fibrous roots and root primordia. Organisms closely resembling *Ps. tumefaciens* came up in the poured plates from three trees; plates from two trees showed small white circular dense colonies not the crowngall organism; and from four trees the plates were sterile. All plates showing bacterial colonies were flooded with sterile water, a suspension made, and this was inoculated into young tomato plants. Inoculations were made from subcultures of colonies resembling

*Ps. tumefaciens* and from suspensions of the macerated tissue of the overgrowth, but all failed to produce crown gall on tomato plants.

Isolations were next made from four trees of the Winesap variety. Well developed overgrowths, resembling callus knots, were found on the main or lateral roots. From these overgrowths an abundance of fibrous roots developed. In three cases, the knots were soft, but firm in texture and with a definite layer of bark. The surface was wrinkled and brown, also suggestive of the common hairy-knot type of hairyroot. The fourth overgrowth was a hard knot, with a diameter and elevation of three-fourths inch, which had developed around a small lateral from the main root. The surface was slightly convoluted. From this overgrowth *Ps. tumefaciens* was recovered, but from no other specimens.

A lot of two-year-old grafted apple trees, variety Sweet Russet, showing overgrowths without a bark layer, bearing an abundance of fleshy roots, was collected at an Iowa nursery in 1928. The overgrowths averaged three-fourths inch in diameter, with an elevation of one-half inch. The surface of the overgrowth was finely convoluted and the tissue soft to a depth of one-half inch. The swelling in every case was located on the scion, apparently resulting from injury in cultivation. The fleshy roots were somewhat flattened. In some cases fibrous roots of normal appearance also arose from the overgrowth (Plate IB).

Using crystal violet bile agar and following the customary procedure, isolations were made from the specimens. In dilution plates from 15 of the specimens, organisms were recovered having the appearance of young colonies of *Ps. tumefaciens* in plate culture. Plates from the other five specimens remained sterile. In streak cultures made from the different plates, the growth at first resembled *Ps. tumefaciens*, but soon became white on potato dextrose agar slants.

In the trials with the organisms recovered from the woolly knot type of overgrowth the routine inoculations were made into young rapidly growing tomato plants. This host was employed, after obtaining galls in all cases from inoculations into tomato plants of *Ps. tumefaciens*, which had been isolated at various times from apple, dahlia, raspberry, rose, incense cedar, peach, hop, poplar, willow, *Rumex crispus*, rhubarb and other plants. Seigler (14), however, was unable to obtain infection on tomato plants with the apple strain of the crown gall organism, but when inoculated into sugar beet, small galls with numerous roots were obtained. The peach strain of the organism induced galls upon both tomato and sugar beet.

Inoculations with 14 organisms closely resembling *Ps. tumefaciens* obtained from the hairy-knot overgrowths were therefore made into sugar beets. A detailed study of the morphological, cultural and serological characters of these organisms was also made by Patel (9). Neither galls nor hairyroot developed from any of the inoculations.

Inoculations were made also into young tomato and sugar beet plants. On certain ones of the tomato plants, there appeared a darkened water-soaked area surrounding the inoculation wound, but no galls developed. Inoculations into sugar beets resulted in seven cases (cultures 1544, 1545, 1546, 1548, 1551, 1553, 1555), in the development of small swellings covered with a mass of fine fibrous roots. The check inoculations using two



apple and one raspberry strain of *Ps. tumefaciens* produced galls with no roots.

To summarize the results of the isolations from young grafted apple trees; the crown gall organism was recovered in only one case from 183 trees, typical of those discarded by the nurserymen as affected with hairy-knot. Bacterial colonies closely resembling *Ps. tumefaciens* came up in poured plates from 50 of the trees, but upon inoculation did not produce infection upon young tomato plants.

#### ISOLATIONS FROM NURSERY APPLE TREES AFFECTED WITH FIBROUS HAIRYROOT

For the cultural trials on young apple stock affected with the fibrous form of hairyroot, trees were collected from a nursery in each of the following states: Indiana, Kansas, Michigan, New York and Tennessee. A total of 146 budded and 17 piece-root grafted trees of the varieties, Jonathan, Wealthy, Delicious, Winter Banana, Golden Russet, Black Ben Davis, Gano, Yellow Transparent, Winesap, Stayman, McIntosh and Fameuse, were employed. These trees were two years old, budded or grafted upon American, French or Italian apple seedlings. The material was taken either directly from the field at digging time, or selected later from stock in storage by the contributing nurserymen or by the writers. The trees were shipped to the laboratory, where, unless isolations were made at once, they were placed in cool, moist storage until ready to be used.

The first lot of trees employed in the isolation studies (collected at the time of digging in October, 1925) came from a nursery in Western New York. This lot, consisting of 15 McIntosh and 20 Fameuse two-year-old, budded on French seedlings, was typical of the trees discarded by nurserymen and inspectors because of the fibrous type of hairyroot on the stock.

Isolations were made on April 10, 1926, from the lot of 15 McIntosh trees. The material macerated for dilution plates, consisting of pieces approximately five millimeters square and three millimeters thick, included the bases of three to five fibrous roots. The bases of the clusters of hairyroots, from which they arose at right angles to the main root, were slightly swollen and flattened. An average of four isolations were made from each specimen. In a majority of cases, the hairyroot condition was confined to an area from the crown of the main root downward approximately three inches. From the 15 trees, 76 isolations were made, but the crown gall organism was not recovered in any case.

The second lot, of 20 trees of the variety Fameuse, was employed in isolation trials made on April 19, 1926. In general appearance, these specimens were similar to those of the variety McIntosh. In these specimens, however, there was in many cases a distinct browning of the internal tissue of the bases of the hairy roots. In a few cases there was a brown discoloration of the outer tissue of the main root surrounding the bases of the hairyroot clusters. These discolorations were evident in the field when the specimens were collected, and were regarded by the inspector as evidence of crown gall infection. In no case was the crown gall organism recovered from the brown tissue. From these trees, 90 isolations were made, and *Ps. tumefaciens* recovered in four cases. In all four cases, the isolations were made from the swollen bases of fibrous roots at the crown. There was



no browning of the tissue of the hairyroots or of the root base. From one specimen, which was similar to the four overgrowths positive for crown gall, an organism was recovered and inoculated into young tomato plants. After three weeks, slight swellings appeared at the point of inoculation. Reisolations, made from the suspected gall, failed to produce the crown gall organism. Since it is not unusual for callus tissue to form at the point of inoculation on tomato plants placed under conditions of high humidity, the above case was considered as a non-infectious overgrowth.

On April 5, 1927, further isolation trials were made on a lot of two-year-old trees consisting of five Stayman and two Delicious, budded upon imported French seedlings. These trees were selected by the owner at an Indiana nursery in the fall of 1926 as typical of those discarded because of hairyroot. The apple stock was grown on a light sandy loam. In making observations in the nursery row before digging, it was found that an average of 12 per cent of the trees of the above varieties showed hairyroots developing at the crown and visible above ground. These observations agreed with others made the previous year, and also with statements of the owner as to the high percentage of this type of hairyroot on apple stock in this nursery.

The hairyroot condition in both these varieties was essentially the same as in the trees previously studied. The fibrous roots arose at right angles to the main root and at the crown. At the crown the swellings were much smaller in size and fewer roots arose from them. A typical tree of this lot is shown in Plate IIA.

An average of four isolations was made from each specimen, and dilution plates were poured from the macerated material including the bases of the root clusters, and the tissue of the swelling from which they arose. These trials failed to yield the crown gall organism.

A lot of 17 piece-root grafted Yellow Transparent trees was obtained from a nursery in Tennessee. In these trees, there was no typical hairy-knot at the union. Instead, the union was slightly swollen and an abundance of fine fibrous roots arose from the stock lip of the union and the upper half of the main root. These trees were similar in appearance to those in which the scion is grafted upon a seedling with the fibrous type of hairyroot.

Isolation trials from two trees from the Tennessee nursery gave sterile plates. Those from the 15 remaining trees showed a sparse development of white, dense colonies not resembling the crown gall organism. Inoculations from the suspensions of macerated tissue and the flooded plates failed to produce infection.

Of the isolation trials on 52 budded and 17 grafted apple trees affected with the fibrous type of hairyroot, the results show that the crown-gall organism was recovered in four cases from one lot of budded Fameuse trees taken from the same nursery.

#### ISOLATIONS FROM YOUNG APPLE TREES AFFECTED WITH THE BURR-KNOT TYPE OF HAIRYROOT

During the seasons of 1926 and 1927, specimens of apple trees with the burr-knot form of hairyroot were collected at various nurseries in Iowa, Indiana, Kansas, Michigan, Nebraska and Tennessee. These were taken directly from the field at the time of digging and placed in cool storage at

the laboratory until ready to be used. These included trees of the varieties, Jonathan, Delicious, Winter Banana, Golden Russet, Black Ben Davis, Winesap, Gano, Stayman and Yellow Transparent, budded on American, French or Italian seedlings.

The first lot of budded trees employed in isolation studies consisted of 20 trees, 11 of the variety Fameuse and 9 McIntosh. These specimens differed from any previously employed in that the main root at the crown and for a distance of two to three inches below, was studded with a series of flattened swellings whose elevation was one-eighth to one-fourth inch, and which, in some cases, entirely encircled the root. The flattened overgrowths, having a definite rough bark layer, were thickly covered with fine fibrous roots in clusters of three to ten. As in previous trials, isolations were made from the tissue of the root bases and the swelling from which the roots arose. *Ps. tumefaciens* was recovered from one of the 11 Fameuse trees. In the McIntosh trees the hairyroots arose in clusters of two to five from the crown. There was only a slight swelling and few hairyroots from it. The well-developed flattened overgrowths seen in the Fameuse trees were absent. The crown gall organism was not recovered from any of the McIntosh specimens.

Isolations were made from two lots of eleven and seven two-year-old Yellow Transparent apple trees budded on imported French seedlings. These trees were obtained, from discards at the time of digging, from two nurseries in southern Tennessee. Typical specimens of these trees are shown in Plate IIC. In these specimens the fibrous hairyroots arose in clusters of three to ten, from a slight swelling resembling small burr-knots as described by Swingle (19). The hairyroots were most abundant at the crown, and for a distance of one to three inches below upon the main root. The bases of the fibrous roots were not swollen. In a few cases, aborted, thickened roots arose from the swelling in the same cluster with the fine fibrous roots below ground. At the crown, just above the ground, only the aborted roots arose from the burr-knot-like swelling. An average of five isolations was made from each specimen, but *Ps. tumefaciens* was not recovered in any case. Isolations from a gall on apple produced by artificial inoculation were positive.

A lot of two-year-old budded apple trees was shipped from a Michigan nursery for isolation trials. These trees, 16 of the variety Wealthy, 17 Jonathan, and 10 Delicious, were budded on Italian seedlings. They were selected by the manager of the nursery as typical of those discarded because of hairyroot.

In the Wealthy trees small flat swellings occurred along the entire main root. From these swellings, clusters of five or more fibrous and in a few cases aborted fleshy roots arose in clusters at right angles to the main root. In no case were the bases of the hairyroots swollen. Plate IIIA shows trees typical of this condition. An average of four isolations was made from each specimen. Tissue, including the bases of the hairyroots and a portion of the swelling from which they arose, was used in making the dilution plates. As a check, isolations were also made from a true crown gall on Opatá plum. All the isolations from the hairyroot specimens were negative, but another isolation from a true gall on plum, produced *Ps. tumefaciens* and infection on young tomato plants.

Similar isolation trials were made upon the lot of 17 trees of the variety Jonathan, selected from discards at the same nursery. The hairyroot condition on these was comparable with that on the Wealthy trees previously described.

The fine fibrous roots arose in clusters of five to 20, resembling burr-knots, upon the main root. The swellings were few in number and located at the crown of the tree in some cases, while in others they were found extending below the crown for almost the entire length of the main root, and in some instances almost encircling it. A typical specimen of this lot of trees is shown in Plate IIB. Frequently the small swellings coalesced into a rough, flat, gall-like overgrowth. In two specimens, both fibrous and fleshy roots arose from the swelling. In no case were the bases of the hairyroots fleshy. Isolations were made from three to five places upon each specimen, but the crown gall organism was not recovered. Similar isolations, from naturally infected plum trees, were positive.

In a similar manner, isolation trials were made upon the lot of 10 hairyroot Delicious trees from the same nursery. These trees presented much the same appearance as those of the varieties Jonathan and Wealthy described above. Flat burr-knot-like swellings occurred along the main root from the crown downward to a distance of three inches. From these swellings fibrous roots, without swollen bases, arose in clusters of five to 30. In a few cases, thickened abortive roots were found in the clusters of fibrous roots. These, with the basal tissue, were employed separately in making isolation trials. Although an average of six isolations was made from each specimen, *Ps. tumefaciens* was not recovered. Control isolations were made from a true gall on an apple seedling and the crown gall organism recovered.

Further trials were made from a lot of budded trees collected at digging time at a Kansas nursery. These consisted of 15 trees of five varieties. Isolations were made first from one Gano and four Black Ben Davis trees. These specimens all showed fine fibrous and short, rather thickened abortive roots arising in clusters of five to twelve from burr-knot-like swellings along the main root or at the crown of the tree. The bases of the hairyroots were not swollen. In the case of these trees, the swellings were few in number, usually not more than three or four, averaging about one-half inch in diameter and with an elevation of approximately one-eighth inch. The swelling was covered with a bark layer through which the roots emerged.

The four Delicious trees used in similar isolations were similar in appearance to the hairyroot condition. There were, however, a larger number of burr-knot-like swellings scattered along the entire main root and at the crown below the bud. In many cases the swelling was thickly studded with fibrous roots. Bulbous or swollen bases of the hairy roots were not observed.

One tree of the variety Jonathan was also employed in the isolations. This specimen presented a more advanced stage of the burr-knot-like swelling, which almost completely covered the main root and crown. There were no fleshy or aborted roots, but the swelling was densely crowded with fine fibrous roots, giving the appearance of hairyroot as illustrated by Stewart, Rolfs and Hall (18).



Five trees of the variety Yellow Transparent, completed the lot from which isolations were made. In these specimens, the fine fibrous roots arose in clusters from small flattened swellings resembling burr-knots. In one case similar swellings were noted on the seedling stock above ground, but these were free from roots. The characteristic appearance of the swellings, and the fact that only those underground bore fine fibrous roots, made their similarity to burr-knots all the more striking. This condition had been observed previously in many of the budded hairyroot trees, but was not so evident as in this particular instance. Sixty-three isolations were made from the lot of 15 trees, but the crown gall organism was not recovered in any case. Control isolations from five galled seedlings artificially infected in the field yielded *Ps. tumefaciens* in all instances.

The last isolation trials were made from a lot of two-year-old budded apple trees consisting of three Stayman Winesap and five Delicious taken from an Indiana nursery. These showed the typical burr-knot form of swellings at the crown and upper two-thirds of the stock, from which arose an abundance of fibrous roots in clusters. Although poured plates from five of the trees showed bacterial colonies, they were not typical of the crown gall organism.

Thus from 107 specimens of budded two-year-old nursery apple trees showing the burr-knot type of hairyroot, the crown gall organism was recovered in only one case. No studies were made upon the organism resembling *Ps. tumefaciens* in the isolation plates, but which failed to produce infections upon young tomato plants. It was assumed for the purpose of these studies that such were not the crown gall pathogen.

#### ISOLATIONS FROM YOUNG BUDDED QUINCE TREES

On March 24, 1927, ten orange quince trees were sent by an Iowa nurseryman to Dr. Melhus for laboratory examination. These trees, two years old and budded upon rooted quince cuttings from France, had been rejected in an interstate shipment as affected with hairyroot. Typical specimens of the lot are shown in Plate IIIB. In these trees clusters of ten or more thickened fibrous roots arose from a slight swelling at the crown just below the bud. The bases of the roots were not swollen. The trees otherwise appeared normal, showing a good root system and well developed trunks. The swellings resembled small burr-knots in outward appearance.

The specimens were thoroughly scrubbed with a stiff brush and the fibrous roots cut off close to the crown. After flaming the surface with alcohol, tissue from the swelling, including the bases of the root cluster, was removed aseptically. After macerating in sterile water the suspension was left to stand for two hours to allow any bacterial organism to diffuse out of the macerated tissue. Dilution plates were made in bile-crystal violet agar, from an average of four pieces of tissue from each specimen. After two weeks, poured plates from six of the specimens remained sterile. From the four remaining specimens, bacterial colonies appeared in the plates. These, for the most part, were small, circular, dense, white, slightly raised, with a flat surface, and not to be confused with colonies of *Ps. tumefaciens*. In one plate, in addition to the above described colonies, others appeared having a closer resemblance to the crown gall organism. These colonics were



translucent, circular, 2-4 millimeters in diameter, but with a darker center and greater density than those of *Ps. tumefaciens*.

Inoculations were made into young tomato plants from subcultures of colonies resembling the crown gall organism and also from the macerated tissue from each specimen. In addition, a suspension was made, by flooding the plates showing bacteria from each specimen, and this was inoculated into young tomato plants. All inoculations failed to produce infection. Control isolations from a small crown gall on tomato yielded the pathogen in practically pure culture.

A second lot of ten two-year-old budded Champion quince trees was selected from the nursery storage as typical of the hairyroot condition found on the preceding quince specimens. These specimens were similar in all respects to those previously employed in isolation trials. After preparing the specimens as before, poured plates were made on April 2, 1927, from an average of four isolations from each tree. Plates from four of the specimens were sterile; from four others only a few white, dense, spreading, bacterial colonies appeared at the edge of the plates, in all probability they were contaminations; from two specimens, colonies resembling *Ps. tumefaciens* came up in six days. Three series of inoculations, from flooded plates, macerated tissue, and subcultures of the colonies resembling those of the crown gall pathogen were made. All inoculations failed to produce infection.

#### HAIRYROOT DEVELOPMENT ON QUINCE CUTTINGS

It is well known to nurserymen and propagators that certain woody plants readily strike roots from cuttings. Each year large quantities of rooted quince cuttings are lined out in the nurseries for the purpose of budding. Examination of the cuttings shows the development of roots in clusters of three or more from the nodes below ground. This suggested that the hairyroot condition on the two-year-old quince trees examined, might be a continuation of growth of the root clusters on the one-year-old cutting.

Therefore, an attempt was made to induce rooting from quince cuttings. Fifteen one-year-old quince cuttings were thoroughly washed and disinfected in 1-1000 mercuric chloride solution and placed in fresh, moist sphagnum. When they had been in the moist sphagnum about four weeks, the cuttings were removed and the development of new roots noted. In every case, the new, slightly fleshy roots arose in clusters of two or more from the node. Plate IVA and C shows both typical rooted quince cuttings as taken from the nursery ready for planting and similar cuttings after storage in moist sphagnum.

This experiment indicates that the production of roots in clusters is a natural habit of the quince. Isolations were made, from three of the specimens in moist sphagnum, to determine whether or not the crown gall organism in the nodal tissue had caused the development of the fleshy roots. The isolations were made from the nodal swelling, including the bases of the root cluster.

From one of the specimens all the plates remained sterile. From one other only a few scattered, dense, white, circular colonies unlike *Ps. tumefaciens* appeared. From the third specimen colonies resembling the crown gall organism appeared in some of the plates. Inoculations from sub-

cultures of the suspected organisms, from suspensions of the flooded plates, and macerated tissue failed to produce galls on young tomato plants.

#### HAIRYROOT ON APPLE SEEDLINGS

Field experiments in 1925 (7) showed that the hairy-knot form of overgrowth was almost entirely confined to those seedlings directly inoculated with *Ps. tumefaciens*. The fibrous type of hairyroot occurred on seedlings grown on steamed and unsteamed soil, independent of wounding or of soil infestation or artificial inoculation with the crowngall pathogen.

Further experiments in 1926 were carried out under field conditions with seedlings from non-disinfected seed grown in unsteamed soil. In these experiments, an attempt was made to determine the extent to which *Ps. tumefaciens* was associated with overgrowths and hairyroot upon seedlings grown in artificially infested and non-infested soil.

Five rows were selected from a field of several acres of seedlings grown on a clay loam. The experimental rows were taken from within the field so that several rows of seedlings not treated lay on both sides. The seedlings in the experimental rows were treated as follows:

- Row 1—Seedlings wounded and inoculated with *Ps. tumefaciens*.
- Row 2—Seedlings wounded. Check.
- Row 3—Seedlings wounded, soil infested with *Ps. tumefaciens*.
- Row 4—Seedlings not wounded, soil not infested. Check.
- Row 5—Seedlings not wounded, soil infested.

The seedling inoculations and soil infestation were made August 4 to 6 and the plants dug November 12, 1926. The inoculations were made by removing the soil to a depth of two inches from the base of the plants, wounding with a scalpel about one and one-half inches below the crown, and applying the inoculum to the fresh wound with a camel's hair brush. Immediately after inoculation the soil was replaced about the plants. The seedlings wounded but not inoculated were treated in a similar manner, but no inoculum was applied to the fresh cut. Soil infestation was accomplished by pouring a diluted suspension of the organism, grown in dextrose bouillon, along the rows at the bases of the plants. In case the plants were wounded, the suspension of the organism was poured along the row after the wounds had been made.

After digging, the seedlings were classified as to the presence or absence of overgrowths alone, overgrowths with or without hairyroots, and the presence or absence of hairyroots in the absence of any overgrowths. These data are given in table 3.

The results of this experiment show that in only one treatment were overgrowths without hairyroot produced, and that was on the seedlings directly inoculated with the crowngall pathogen (plot 1). The fact that only seven per cent of the seedlings inoculated directly with *Ps. tumefaciens* developed galls or overgrowths, indicates that they are not readily susceptible to crowngall infection under field conditions. These results are in accord with those obtained in previous field experiments (7) upon apple seedlings in steamed soil. It is also seen that 13 or 8.3 per cent of the 155 overgrowths produced upon the artificially inoculated seedlings bore fibrous hairyroots, but fleshy hairyroots were absent. The fleshy hairyroots oc-

TABLE 3. *Overgrowths and Hairyroot on Seedlings in Unsteamed Soil.*

Plot	Treatment	Total Seedlings	Overgrowth only		Overgrowths with hairyroot				Hairy-root only		Clean	
			Number	Per cent	Fibrous		Fleshy		No.	Pct.	No.	Pct.
1	Wounded and inoculated with <i>Ps. tumefaciens</i>	2208	155	7.02	13	0.58	0	0	35	1.58	2005	90.81
2	Wounded only. Check	2475	0	0	0	0	11	0.44	53	2.14	2312	93.42
3	Wounded. Soil infested	2486	0	0	0	0	10	0.40	61	2.45	2325	93.53
4	Not wounded. Soil not infested	2355	0	0	0	0	0	0	46	1.95	2309	98.05
5	Not wounded. Soil infested	2226	0	0	0	0	3	0.13	13	0.58	2210	99.29

curred upon overgrowths on seedlings in plots two, three and five. In plot two, the seedlings were wounded but not inoculated; in plot three, they were wounded and the soil infested; in plot five, the seedlings were neither wounded nor inoculated, but the soil was infested. From these results it appears that the production of fleshy hairyroots from an overgrowth has little significance with regard to infection by the crowngall organism.

The occurrence of fibrous hairyroots in the absence of overgrowths on the seedlings is rather uniformly distributed throughout the plots with no evidence of being influenced by direct inoculation or soil infestation by the pathogen. Thus, in plot one, where the seedlings were directly inoculated, only 1.5 per cent were affected; in plot two, without treatment of the soil or plants, 2.1 per cent of the seedlings showed fibrous hairyroot.

#### ISOLATION STUDIES ON APPLE SEEDLINGS

In the field experiment, counts made at the time of digging showed that overgrowths occurred upon seven per cent of the seedlings directly inoculated with the crowngall organism. At this time, it was not possible to determine whether or not all the overgrowths resulted from crowngall infection. Subsequently isolations were made from the seedlings with overgrowths taken from plot one. In preparing for shipment, part of the lot was misplaced at the nursery and discarded as culls. The remaining 25 was shipped to the laboratory and placed in cool storage until ready for use.

#### *Isolations from Overgrowths*

On December 21, 1926, isolations were made from overgrowths on 17 seedlings; the remaining eight were used for anatomical studies. With one exception, these galls were soft and spongy in texture, with a smooth surface and with no bark layer or roots. On cutting, the interior tissue oxidized rapidly, becoming reddish brown. The size of the galls varied somewhat, however, averaging 15 millimeters in diameter, with an elevation of five millimeters. In the exception noted, the tissue of the overgrowth was hard and woody, with a true bark layer and a few roots. In every case, the galls arose from some point along the incision made during the process of inoculation. An average of five isolations was made from each gall. Two of the specimens yielded only small, white, dense, flat-surfaced colonies not resembling *Ps. tumefaciens*. From one specimen all plates were sterile. Colonies closely resembling *Ps. tumefaciens* came up in the plates from the other 13 seedlings. Only seven specimens, however, yielded the crowngall organism as evidenced by infection upon young tomato plants. In each case positive isolations were made from the galls of spongy texture, lacking a bark layer and roots; the hard woody overgrowth with roots failed to yield the pathogen. *Ps. tumefaciens* was not recovered from galls which had started to decay.

#### *Isolations from Hairy-Knots*

At different times during the period December 8, 1926, to January 4, 1927, isolations were made from the seedlings, showing hairyroots associated with overgrowths and hairyroot alone, which were taken from the experimental plots.



Isolations trials were next made from the lot of 10 specimens (table 3) from plot three in which the soil was infested after wounding the seedlings. These seedlings all showed the hairy-knot form of overgrowth. In eight cases the overgrowth bearing fibrous roots arose above, below, or on the side opposite the wound. In two specimens the overgrowth arose from the meristematic tissue of the wound. The overgrowths were small, averaging one centimeter in diameter and with an elevation slightly less. Fine fibrous roots developed in abundance from the overgrowth. The entire swelling was macerated in making the isolations. The crowngall organism was recovered from only one specimen. In this seedling, the gall arose from the wound made on the root just before the soil about the base of the plants was infested with the pathogen. The small percentage of true crowngalls in this plot indicates that wounding of the seedling when grown in infested soil may occur without attendant infection.

In plot two there were 11 seedlings with fleshy roots arising from small overgrowths just below the crown. The seedlings in this plot were wounded, but not inoculated; the soil was not infested. Isolations from these specimens failed to yield the crowngall organism, but in control isolations from a true gall produced on an apple seedling by artificial inoculation, *Ps. tumefaciens* was recovered. The knots were small with a wrinkled surface, averaging 1.5 centimeters in diameter and an elevation of one centimeter, from which arose an abundance of small, slightly fleshy roots. The overgrowths in eight cases were not associated with the wound, but in three specimens they arose from the wound issue.

In plot five overgrowths with fleshy roots developed upon three seedlings. In this plot the seedlings were not wounded, but the soil was infested. The hairyroots in two cases arose from the callus tissue developed at the end of a broken lateral root. In the other case, the overgrowth developed from a wound just below the crown. These knots were small in size, averaging one centimeter in diameter, with an elevation of about one-half centimeter. Isolations made from the three seedlings above described failed to yield the crowngall pathogen. In control isolations from galls produced by artificial inoculation on four apple seedlings the pathogen was recovered in three cases.

The results obtained from the trials show that the presence of fleshy roots arising from an overgrowth cannot be regarded as evidence of infection by the crowngall organism.

#### *Isolations from Seedlings with Hairyroot in the Absence of Overgrowths*

As shown in table 3, the largest percentage of abnormal seedlings in the experimental plots included those in which fine fibrous hairyroots occurred in the absence of a well developed overgrowth. It is further seen that this type of hairyroot was rather evenly distributed among the plots, apparently independent of the earlier treatment of the seedlings or of soil infestation. This appears to be the type of hairyroot most commonly found on young budded apple trees in the nursery. The fact that seedlings so affected are sometimes discarded by the nurserymen, as supposedly infected with *Ps. tumefaciens*, led to further isolation trials from the seedlings showing hairyroot in the absence of a well developed overgrowth, as is typified by the hairy-knot form.

Two distinct forms of hairyroot were present in these seedlings. The first, Plate IVD, was characterized by the presence of clusters of three to ten fine fibrous roots arising directly from the upper third of the main root or from the crown in the absence of any swelling. The bases of the fibrous roots showed no evidence of being swollen. The roots themselves appeared normal except for their abundance within a small area. In some cases larger lateral roots arose from among the fine fibrous roots. This type of hairyroot will be referred to as the fibrous form.

In the second, or burr-knot type, Plate IVB, clusters of fine fibrous roots arose from the depressed center of a flattened circular swelling. These swellings rarely had an elevation of more than three millimeters above the main root or crown upon which they were borne. In appearance the swellings closely resembled the flattened type of burr-knot as illustrated by Swingle (19). In rare cases, the coalescence of the swellings resulted in the upper third of the main root being surrounded with hairyroots. This type of hairyroot, while present on seedlings, was more frequently seen on the two-year-old budded trees (Plates IIB, C, and IIIA).

#### *Isolations from the Fibrous Type of Hairyroot*

For the isolation trials, 65 seedlings showing the fibrous form of hairyroot were available. The first lot from which isolations were made on December 15, 1926, consisted of 12 seedlings. The fibrous hairyroots arose in clusters of two to ten from the crown of the seedling. In only one case were the bases of the roots slightly swollen. In other respects the seedlings appeared normal, that is, the main root showed no evidence of hairyroot development and was of average diameter and length. In making the isolations an average of three pieces of tissue including the bases of at least three fibrous roots were taken from each specimen. The crowngall organism was not recovered from any of the specimens. Control isolations made from galls on five artificially inoculated apple seedlings resulted in the recovery of *Ps. tumefaciens* in three cases.

On December 21, 1926, similar isolations were made from a lot of nine seedlings showing the fibrous form of hairyroot. These seedlings were similar in all respects to those from which isolations were made on December 15. In every specimen, the fine fibrous roots arose in clusters of two to eight from the crown of the seedling. None of the hairyroots had swollen bases. Control isolations were made from true galls on two budded, two-year-old apple trees, naturally infected and from two seedlings inoculated with the crowngall organism. *Ps. tumefaciens* was not recovered from any of the hairyroot specimens, but three of the four controls yielded the pathogen. On December 22, isolations were made from a third lot of 13 seedlings showing the fibrous type of hairyroot. On eight of the specimens, the clusters of two to ten fine fibrous roots arose from the crown of the plant; in five of the seedlings they arose from the main root. The seedlings otherwise appeared normal. None of the hairyroots showed swollen bases. Control isolations were made from a seedling gall produced by artificial infection. All isolations from the hairyroot seedlings failed to yield the pathogen. From the control isolation the crowngall organism was recovered.

On December 28, 1926, isolation trials were made on a lot of 16 hairyroot seedlings of the same type as previously employed. In addition, control isolations were made from three soft overgrowths on artificially infected seedlings and from the bases of small lateral roots on two normal seedlings. The clusters of three to five fine fibrous roots arose from the crown on 12 seedlings, from the upper third of the main root on two seedlings and from the crown, and immediately beneath it on the main root on two seedlings. All attempts at isolation of the crown gall organism from the hairyroot and normal seedlings were negative. Of the three artificially infected controls, one yielded the pathogen.

Further isolations were made from a lot of 15 fibrous hairyroot seedlings on January 4, 1927. On eight seedlings, the clusters of three to eight hairyroots arose from the crown and also directly below on the main root; on six, from the crown alone, and on one only from the main root. Some of the hairyroots showed swollen bases. In five cases apparently normal lateral roots arose from the hairyroot clusters. Control isolations were made from a true gall on a naturally infected Opata plum root. None of the hairyroot seedlings yielded the crown gall organism, but it was recovered in abundance in the control isolations.

On January 12, 1928, isolations were made from a lot of 40 seedlings showing the fibrous type of hairyroot in the absence of any overgrowths. In these specimens, the fine fibrous roots arose in clusters of three to five, at right angles from the main root. In three cases, fleshy roots also occurred in the same cluster with the fibrous roots. There was no indication of an enlargement at the bases of the hairyroot clusters. After surface disinfection by flaming, isolations were made using the bases of the root clusters and the root tissue immediately beneath. *Ps. tumefaciens* was not recovered from any of the specimens. Isolations also were made from seven normal seedlings and from a crown gall on Bolleana poplar. The crown gall organism was recovered only from the gall on Bolleana poplar.

#### *Isolations from Burr-Knot Type of Hairyroot*

The burr-knot type of hairyroot (Plate IVB) occurred on a few seedlings in the experimental plots. In this type of abnormality the clusters of fine, fibrous roots arise from the depressed center of a slightly elevated, flattened, circular swelling at the crown or upon the upper portion of the main root. This form of hairyroot is commonly seen on the crown and main root of young nursery trees budded upon French, American or Italian seedlings. The fact that seedlings frequently are lined out for budding stock with little sorting suggests that this form of hairyroot may carry over from the lining out stock rather than from external factors affecting normal seedlings after budding. Attempts, therefore, were made to determine by isolation trials, the relation of this form of abnormality to crown gall infection. For the first trials only 18 seedlings were available. In these specimens clusters of three to ten fine fibrous roots arose from a sunken circular area, three to eight millimeters in diameter, with a slightly swollen rim. The appearance of the clusters of roots arising from the rough flattened swelling was similar to that of a small burr-knot. The hairyroot bases were not swollen. From practically all the hairyroot clusters, one or more appar-



ently normal laterals arose. In four specimens, the tissue beneath the base of the hairyroot clusters was brown. The hairyroot cluster occurred at the crown and immediately below on the main root on 12 specimens, at the crown alone on three, and on the upper portion of the main root in three cases. Three to five isolations were made from each specimen, all of which failed to yield the crown gall organism. Of the six control isolations from apple seedling galls produced by artificial infection, four yielded *Ps. tumefaciens*.

On January 24 and March 15, 1928, further isolations were made from lots of seven and four seedlings showing the burr-knot type of hairyroot. These were taken from lots of seedlings showing the fibrous type of hairyroot, which had been culled out by the nurserymen when the stock was being graded. In these seedlings the fine hairyroots were fibrous in character and without enlarged bases.

Four of the plates made from the lot of seven seedlings developed colonies similar in appearance to that of the crown gall organism. However, no infection resulted upon repeated inoculation into young tomato plants. In all the plates from the second lot of four seedlings, colonies resembling *Ps. tumefaciens* developed, and with one exception, upon inoculation failed to produce typical galls either on young tomato plants, marguerite (*Chrysanthemum frutescens* L.), apple, or on sugar beet. From one plate, however, colonies were streaked (culture 1207) which were used to inoculate the bases of young shoots on apple grafts and the crown and root of the sugar beet. On four of the five apple shoots inoculated, a slight swelling bearing fleshy roots developed. On the two sugar beets, an abundance of fibrous roots developed from the point of inoculation.

Further isolations from a similar lot of 19 seedlings showing the burr-knot type of hairyroot were made on June 5. These were selected by the senior writer at the time of digging in November, 1927. In plates from six of the seedlings, colonies resembling *Ps. tumefaciens* developed. The colonies in the other plates had no resemblance to the crown gall organism. Repeated inoculations into young tomato plants failed to produce infection. Further inoculations were made into apple and sugar beet. Streaks of colonies from two of the seedlings, when inoculated into young apple shoots, resulted in the development of small swellings with fleshy roots. From subsequent inoculations into sugar beets, both gall and fibrous hairyroots were induced by two cultures (1289, 1294), the other four failed to produce any overgrowth or root formation.

It is of interest to note that the three cultures which reacted with apple and sugar beet, when grown for some time on slants, slowly lost the close resemblance to *Ps. tumefaciens*, a characteristic shown during their early growth and in the plate cultures. Since overgrowths quite frequently develop on the scions of grafted apples as the result of repeated puncturing with a sterile needle, it is not clear whether or not the formation of the swelling is entirely a result of inoculation. From the results of inoculations with several strains of the crown gall organism, the development of fleshy roots from the apple, and fibrous roots from the sugar beet is not considered a typical reaction of *Ps. tumefaciens*. Further studies were carried out to determine the relation of these organisms to *Ps. tumefaciens*.



## MORPHOLOGICAL AND CULTURAL STUDIES OF HAIRYROOT ORGANISMS

In the isolation trials upon apple seedlings showing the burr-knot type of hairyroot and upon young grafted apple trees with fleshy roots arising from overgrowths upon the scion, 10 organisms were isolated which closely resembled young colonies of *Ps. tumefaciens* in plate culture. Subsequent inoculations into young apple shoots, sugar beet plants and stems of *Chrysanthemum frutescens* resulted in the development of an abundance of fleshy roots from overgrowths on the apple (Plate VA), clusters of apparently normal lateral roots from slight swellings on the sugar beet (Plate VB), and clusters of aborted roots from slight swellings on *Chrysanthemum frutescens* (Plate VIA). All inoculations into the tips of young tomato plants failed to induce either overgrowths or roots. In control inoculations with strains\* of *Ps. tumefaciens* into young apple, sugar beet and tomato plants typical galls without hairyroots developed.

It was also noted that the agar plate colonies of the organisms recovered from the hairyroot specimens, although at first closely resembling *Ps. tumefaciens*, gradually became less translucent. On agar slants the growth also became white instead of the tawny color characteristic of the strains of *Ps. tumefaciens*.

Because of the differences between the symptoms induced by these organisms, and those induced by the raspberry and apple strains of the crown gall pathogen when inoculated into apple, sugar beet and tomato plants, and the difference in the growth of the organisms on agar slants, it seems advisable to make further morphological and cultural studies of the organisms isolated from the hairyroot specimens. Two organisms isolated from fleshy hairyroot on nursery apple trees (cultures 1544 and 1555), and three others (cultures 1207, 1289 and 1294) isolated from the burr-knot type of hairyroot on apple seedlings, previously mentioned, were employed in the cultural studies. In addition, five strains of *Bacillus radiobacter*\* (cultures 1455, 1456, 1458, 1460 and S) and seven strains of *Pseudomonas tumefaciens* (cultures 468, 1219, 1227, 1228, 1236, 1303, R) isolated from various hosts were carried as checks. The morphological and cultural characters of the seven strains of *Ps. tumefaciens*, five hairyroot organisms, and five strains of *B. radiobacter* are given in table 4.

The following method of purification of the cultures was employed. Inoculation of the organisms was made from a young culture on potato dextrose agar into neutral peptone dextrose broth. After 24 hours growth, dilutions were made using a 3 mm. loop in five 10 c.c. tubes of peptone dextrose broth. After 24 hours growth, four dilution agar plates were poured from the fifth broth culture. Streaks were made, after 72 hours, from single colonies in the fourth dilution agar plate. With the streak

---

\*In this paper the word strain is used to denote cultures of organisms isolated at different times or from different hosts. It is not used in a physiological sense.

\*Cultures of *B. radiobacter* numbers 1455, 1456, 1458 and 1460 were obtained from the United States Department of Agriculture, Bureau of Chemistry and Soils, through the courtesy of Dr. N. R. Smith. Culture S was supplied by Dr. F. B. Smith, Department of Farm Crops and Soils, Iowa State College.

Cultures of *Ps. tumefaciens* numbers 1219, 1227 and 1228 were obtained from Dr. A. J. Riker, University of Wisconsin.

TABLE 4. Morphological and Cultural Characters of Seven Strains of *Ps. tumefaciens*, Five Hairyroot Organisms and Five Strains of *B. radiobacter*.

Cul- ture No.	Size of rod		Motility	Gram Stain	Acid fast	Cap- sules	Spores	Chromo- genesis	Production of			Voges- Pros- kauer test	Methyl red test	Nitrate reduc- tion	Methy- lene blue reduc- tion	Milk coagu- lation	Milk dige- stion	Litmus milk browned	Growth in			Gelatin lique- faction	Dia- static action on starch	Thermal death point
	Length	Width	Flagella						H <sub>2</sub> S	Indol	NH <sub>4</sub>								Cohns solu- tion	Uschin- sky's so- lution	Fermi's solu- tion			
468	1.5-2.9	0.6-0.8	Polar	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	53°C
1219	1.4-3.0	0.6-0.8	do	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
1227	1.5-3.0	0.6-0.8	do	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
1228	1.3-2.6	0.6-0.8	do	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
1236	1.5-3.0	0.6-0.9	do	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
1303	1.5-3.1	0.5-0.8	do	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
R	1.5-2.1	0.6-1.0	do	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	
1207	1.6-2.8	0.6-0.8	Peritrichous	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	Above 53°C
1289	1.9-3.0	0.6-1.0	Peritrichous	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
1294	1.6-2.7	0.6-1.0	do	—	—	—	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
1544	1.1-2.2	0.4-0.75	Polar	—	—	—	—	—	—	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
1555	1.5-2.2	0.4-0.7	do	—	—	—	—	—	—	—	+	—	—	—	+	—	+	+	—	—	+	—	—	
1455	1.8-3.0	0.6-1.0	Peritrichous	—	—	+	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	50°C
1456	1.1-2.0	0.4-0.7	Peritrichous	—	—	+	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	Above 53°C
1458	1.4-2.0	0.4-0.8	do	—	—	+	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
1460	1.3-2.4	0.4-0.7	do	—	—	+	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do
S	1.5-3.0	0.5-1.0	do	—	—	+	—	—	+	—	+	—	—	—	+	—	+	+	—	—	+	—	—	do

TABLE 5. Reactions of Seven Strains of *Ps. tumefaciens*, Five Hairyroot Organisms and Five Strains of *B. radiobacter* in Broths Containing Various Carbohydrates, Alcohols and Glucosides.

Cul- ture No.	Galac- tose	Dex- trose	Saccha- rose	Raffin- ose	Gly- cerol	Arabi- nose	Xy- lose	Mal- tose	Lactose	Dex- trin	Manni- tol	Levu- lose	Sali- cin	Inu- lin
468	S-A1	S-A	S-A1	S-A1	A1	S-A1	S-A1	O	A1	A1	S-A1	S-A1	A	S-A1
1219	O	S-A	S-A	S-A1	A1	O	S-A1	S-A	A1	A1	S-A1	S-A1	A	A1
1227	O	S-A	O	S-A1	A1	S-A1	S-A1	O	A1	A1	A1	S-A1	A	A1
1228	S-A1	S-A1	S-A1	S-A1	A1	S-A1	S-A1	S-A	A1	A1	A1	S-A1	A	S-A1
1236	O	S-A1	S-A1	S-A1	A1	S-A1	S-A1	A1	A1	A1	A1	A1	A1	A1
1303	A1	A1	A1	A1	A1	A1	S-A1	A1	A1	A1	S-A1	S-A1	S-A	A1
R	S-A1	S-A1	S-A1	A1	A1	S-A1	S-A1	S-A1	A1	A1	S-A1	S-A1	S-A	S-A1
1207	A	A	A	A	A1	S-A1	S-A1	A	A	A	S-A	A	A	S-A1
1289	S-A1	S-A1	A1	O	O	A1	S-A1	A1	A1	S-A1	S-A1	S-A1	S-A1	S-A1
1294	A	A	A	A	A1	A	O	A	A	A	A	A1	A	S-A1
1544	A1	A1	A1	A1	A1	A1	A1	A1	A1	A1	A1	A1	S-A1	A1
1555	A1	A1	A1	A1	A1	A1	A1	A1	A1	A1	A1	A1	S-A1	A1
1455	S-A	A	O	S-A1	S-A1	S-A	S-A1	O	S-A1	S-A1	O	S-A1	S-A	S-A1
1456	O	S-A	O	S-A1	O	O	S-A1	O	S-A1	A1	S-A1	O	A	S-A1
1458	S-A	A	O	S-A1	O	S-A	S-A1	O	S-A1	S-A1	O	S-A1	A	S-A1
1460	S-A	S-A	O	S-A1	O	S-A	O	O	S-A1	S-A1	S-A1	O	S-A	S-A1
S	O	S-A	O	O	O	S-A	O	O	S-A1	S-A1	S-A1	O	S-A	S-A1

O=No change in reaction of medium.  
A= Acid reaction.  
A1= Alkaline reaction.  
S= Slight.





cultures so obtained, the broth and poured plate dilutions as given above were repeated twice. Subcultures of the organisms obtained from the fourth plates in the third series of dilutions were then used to inoculate young sugar beets to prove their pathogenicity. In the cultural trials, this method of purification of the cultures was employed for each series of tests made.

#### MORPHOLOGY

All the organisms were readily stained without heating with Ziehl's carbol fuchsin, Loeffler's methylene blue, and gentian violet.

There was a certain amount of variation in the size of the organisms in the 24 hour cultures. The organisms of the strains of *Ps. tumefaciens* measured  $1.5$  to  $2.5\mu$   $\times$   $0.6$  to  $0.75\mu$  and occurred mostly as single rods, sometimes in twos, but rarely in threes. The hairyroot organisms measured  $1.0$  to  $3\mu$   $\times$   $0.4$  to  $1.2\mu$ , occurring usually as single rods, but frequently in chains of two, three, and rarely four. The strains of *B. radiobacter* averaged  $1.1$  to  $3.0\mu$   $\times$   $0.3$  to  $1.0\mu$  and occurred singly.

Motility of the organism was observed in 18 to 24 hour cultures by the hanging drop method. With Casares-Gil's stain, flagella were also demonstrated in six to 72 hour cultures. The flagella of the strains of *Ps. tumefaciens* (Plate VIIA) were, for the most part, unipolar, although in a few cases single organisms showed bipolar single flagella. In a few cases, single organisms showed two unipolar flagella. The five strains of *B. radiobacter* showed peritrichous flagella, usually four or more in number (Plate VIID and E). Of the hairyroot organisms cultures 1207, 1289 and 1294 showed peritrichous flagella (Plate VIIB, C, F). In cultures 1544 and 1555 the flagella were unipolar and borne singly. Over 50 slides were made of these cultures at ages six to 48 hours, but in no case was the peritrichic condition demonstrated. In respect to their flagellation, cultures 1544 and 1555 were identical with those of the eight strains of *Ps. tumefaciens*.

The strains of *B. radiobacter* were readily differentiated from the other organisms by means of the almost unstained capsule surrounding the majority of the organisms, from three days or older cultures, in the water of syneresis on potato dextrose agar slants (Hiss' method).

All of the organisms from 24 hour cultures were non-acid fast and gram-negative. In six day old cultures none of the strains of *Ps. tumefaciens*, *B. radiobacter* nor the hairyroot organisms formed spores.

In two to ten day old cultures, the star shaped bodies formed by the radiation of the organisms from a common center are quite characteristic of all the strains of *B. radiobacter* employed. This character of growth is well illustrated by Löhnis and Hansen (5) and is used by them as one of the chief means of differentiating *B. radiobacter* from strains of the legume nodule organism.

#### CULTURAL CHARACTERS

*Nutrient Beef Broth.* After three days, growth of all the organisms in nutrient beef broth was abundant with the formation of slight sediment. With few exceptions, a pellicle was formed with moderate clouding of the bouillon. No pellicle was formed in the following cultures of hairyroot organisms: numbers 1289 and 1544.



*Potato Dextrose Agar Dilution Plates.* On potato dextrose agar plates, colonies of *Ps. tumefaciens* appear in 24 to 48 hours. The colonies are one to two millimeters in diameter, convex, shining, translucent, with entire margin, and usually with a slightly denser brownish center.

With two exceptions, growth of the hairyroot organisms was equally as rapid and showed the same characteristics. Two of the hairyroot organisms were slower in growth both in plates and in streak cultures. The strains of *B. radiobacter* showed practically identical growth characters in young cultures. However, growth after 72 hours appeared slightly more rapid, the colonies becoming more dense than either those of *Ps. tumefaciens* or the hairyroot organisms.

*Beef Agar.* On beef agar plates growth of all the organisms was slightly less rapid than on potato dextrose agar. The colonies of *Ps. tumefaciens*, after 48 hours, were one to two millimeters in diameter, convex, translucent, with slightly more dense centers, and entire margins. Those of the hairyroot organisms were of approximately the same size and shape although they were slightly more dense throughout. The strains of *B. radiobacter* grew more rapidly than either of the other groups of organisms, but with the same general growth characteristics.

*Agar Stabs.* In beef agar stab cultures three days old *Ps. tumefaciens* gave abundant, white, translucent, shining, convex, surface growth. Growth occurred along the entire length of the stab, becoming more scant toward the bottom. The hairyroot organisms gave abundant growth on the surface of the agar. Surface growth in all the hairyroot cultures was translucent, white, shining, smooth and convex. All strains of *B. radiobacter* gave abundant, white, shining, smooth, translucent, convex surface growth. In cultures 1456 and 1458, respectively, growth was evident to a depth of one-half to three-fourths the length of the stab. In the remainder of the cultures there was good growth along the entire length of the stab.

*Agar Slants.* On potato dextrose agar slants the growth of the strains of *Ps. tumefaciens* was filiform at first, gradually spreading over the entire surface of the slant. The bacterial mass in culture was translucent and glistening, becoming slightly tawny with age. The hairyroot organism also gave a filiform growth, the bacterial mass soon becoming white and partially losing its luster. Growth was not so rapid as with *Ps. tumefaciens*. The strains of *B. radiobacter* gave a filiform, shining, translucent growth, rapidly spreading in the water of syneresis at the base of the slant. In older cultures, this basal growth was white and slightly viscid.

On beef agar slants of *Ps. tumefaciens* the streaks were filiform, with entire edges, translucent, shining and white at first, later becoming slightly tawny in color. Growth was abundant. The hairyroot organisms with two exceptions (cultures 1294 and 1207) gave moderate to abundant white, filiform, shining, translucent growth with entire edges. In these two cultures growth was scant, but of the same character as that shown by the other cultures. The strains of *B. radiobacter* showed similar growth reactions. In ten day old cultures, the growth remained white in contrast to the slightly tawny color shown by the strains of *Ps. tumefaciens*.

*Gelatin Liquefaction.* Gelatin liquefaction trials were made, employing Frazier's (3) method for the detection of proteolysis. All of the strains

of *Ps. tumefaciens* and *B. radiobacter* gave negative tests in both series of plates. Of the hairyroot organisms cultures 1289, 1544 and 1555 also gave negative tests. Cultures 1207 and 1294 gave a positive test.

*Milk.* All the organisms tested gave abundant growth in plain milk. The strains of *Ps. tumefaciens*, after 28 days, showed a distinct browning of the medium with partial digestion of the milk, evidenced by a clear zone extending one-fourth to three-fourths the depth of the medium. There was no coagulation of the medium.

In the cultures of the hairyroot organisms there was marked variation in the reactions in milk. Cultures numbers 1289 and 1544 showed no digestion and no browning of the medium. In the remainder, both digestion of the milk and browning of the medium took place. In cultures numbers 1207 and 1294, one-half or less of the milk was digested; in culture 1555 digestion was complete in 37 days. There was no coagulation.

All the strains of *B. radiobacter*, except culture S, showed slight to moderate digestion of milk after 28 days. There was no coagulation of milk in any of the cultures. The color of the medium was changed to yellowish brown.

*Litmus Milk.* All the cultures of *Ps. tumefaciens* showed partial digestion of litmus milk with browning of the medium after 21 days. There was no coagulation of the medium.

The cultures of the hairyroot organisms with one exception (1544) showed partial or complete digestion of the milk. In culture number 1544 there was no digestion after 37 days. However, in all the cultures growth was good with browning of the medium, with the exception of number 1544, in which the milk became blue-gray in color. There was no coagulation of the medium.

In all the cultures of *B. radiobacter*, after 28 days there was slight to moderate digestion of the milk with the medium turning chocolate brown. The color of the medium with the strains of *B. radiobacter* was darker than that with the strains of *Ps. tumefaciens* or the hairyroot organism. There was no coagulation of the milk.

*Methylene Blue in Milk.* There was moderate digestion of methylene blue and of milk with all the cultures of *Ps. tumefaciens*, the medium becoming light brown in color with no coagulation after 21 days.

The cultures of the hairyroot organism also showed moderate digestion of methylene blue and slight digestion of milk with the exception of number 1544. There was no coagulation in any of the cultures nor any browning of the medium after 28 days.

The cultures of *B. radiobacter* showed complete digestion of methylene blue with a slight digestion of the milk but no coagulation of the medium. After digestion of the methylene blue the cream color of the medium became tinged with brown (28 days).

*Action on Starch.* There was no diastatic action on starch (iodine test) shown by any of the cultures of *Ps. tumefaciens*, *B. radiobacter* or the hairyroot organism.

*Ammonia Production.* All cultures of *Ps. tumefaciens*, *B. radiobacter* and the hairyroot organism in nutrient broth gave a positive test for ammonia after 10 days.

*Indol Production.* None of the cultures of *Ps. tumefaciens*, *B. radiobacter* or the hairyroot organism produced indol after 20 days in Dunham's solution.

*Hydrogen Sulphide Production.* With the lead acetate paper test, all the strains of *Ps. tumefaciens* and those of *B. radiobacter* produced hydrogen sulphide in beef bouillon after 12 days. With the hairyroot organisms, hydrogen sulphide was produced in cultures 1207, 1289 and 1294. With the last two cultures there was only a faint browning of the paper. Cultures 1544 and 1555 showed no hydrogen sulphide production.

*Reduction of Nitrates.* The organisms were grown in nitrate broth for two weeks and tests made for the presence of nitrites. There was no reduction of nitrates by any of the strains of *Ps. tumefaciens*, *B. radiobacter* or the hairyroot organisms.

*Carbon Metabolism.* The organisms were grown in one per cent peptone bouillon (pH 7.2) to which one per cent of the following carbohydrates, alcohols and glucosides was added: dextrose, galactose, lactose, saccharose, glycerol, dextrin, raffinose, xylose, arabinose, maltose, mannitol, levulose, salicin and inulin. In order to test the reaction of the medium during bacterial growth, to each liter of media 0.5 c.c. of a one per cent alcoholic solution of brom cresol purple and 0.5 c.c. of a one per cent solution of cresol red was added. By the addition of these indicators, having a pH range of 5.2 to 8.8, the production of alkali or acid is readily detected. This has been found by Conn and Hucker (2) a satisfactory method for determining the changes in the reaction of growing cultures. A small tube 6 cm. x 0.6 cm. with closed end was inverted in each tube of medium and used in place of the usual fermentation tube, to determine the presence of gas and also whether or not the organism would grow anaerobically. The cultures were examined at regular intervals of 48 hours. Growth was abundant in all the carbon compounds tested. The results of duplicate trials after 10 days growth, using three cultures of each organism, are summarized in table 5.

The reaction of the strains of *Ps. tumefaciens* in one per cent peptone broth containing one per cent of the following: galactose, saccharose, raffinose, glycerol, arabinose, xylose, lactose, dextrin, mannitol and inulin was alkaline. In broths with one per cent dextrose and salicin the reaction was acid; with maltose the reaction with two strains was alkaline (cultures 1303 and R); with two strains (cultures 1219 and 1236) it was acid, and in three cases (cultures 468, 1227, 1228) no change in the medium took place.

For the strains of *B. radiobacter* the reaction was alkaline in one per cent peptone broth containing one per cent of the following carbon compounds: saccharose, raffinose, glycerol, xylose, lactose, dextrin, mannitol and inulin. There was no change in the reaction of the medium using maltose. In broths with one per cent of galactose, dextrose, arabinose or salicin, the reaction was acid. Thus the strains of *Ps. tumefaciens* and *B. radiobacter* employed can be separated on the basis of the acid reaction in galactose and arabinose and no change in the reaction of saccharose broth.

The hairyroot organisms were not, as an entire group, consistent in their reactions in the various carbohydrates. They may be divided roughly



into two groups, the one producing an acid reaction (cultures numbers 1207 and 1294) and the other (cultures numbers 1289, 1544 and 1555) in which the reaction is alkaline. It should be noted, however, that the group reaction above given does not apply for each culture in all the carbon compounds employed. With cultures numbers 1207 and 1294, the reaction in peptone broth containing galactose, dextrose, saccharose, dextrin, levulose and salicin was acid. In raffinose, maltose, and lactose the reaction was acid. In glycerol, arabinose, xylose and inulin culture number 1207 showed alkaline reaction; in glycerol, levulose and inulin culture number 1294 showed an alkaline reaction; but in xylose it showed no reaction.

In the alkaline producing group, culture number 1544 behaved consistently with all the carbon compounds while culture number 1289 gave an alkaline reaction in all cases except with raffinose and glycerol, in which the medium was not changed. In respect to their reaction in broth containing the various carbon compounds, these two organisms appear to be closely related to *Ps. tumefaciens*.

It should be noted, however, that there also are variations of certain strains of *Ps. tumefaciens* and *B. radiobacter* from their general group reaction (cultures 1219 and 1227 in saccharose, 1303 in maltose, dextrose and salicin; cultures 1456 and S in galactose and levulose, etc.) and that the degree of acidity or alkalinity varied with the individual cultures. Dealing with several strains of organisms of the different groups it is to be expected that the physiological reactions of any one group would show certain variations.

None of the cultures employed in these trials produced gas nor any growth within the gas tube, indicating aerobiosis.

*Acetyl-Methyl-Carbinol Production.* The production of acetyl-methyl-carbinol by the organism in peptone sucrose broth was determined by the Voges-Proskauer (20) test. In all cases the tests were negative. In the coli-aerogenes group, organisms showing the negative Voges-Proskauer reaction usually give a positive test with methyl red.

The strains of *Ps. tumefaciens* as well as those of *B. radiobacter* and the hairyroot organisms gave a negative reaction with methyl red. The results with strains of *Ps. tumefaciens* are in accord with those of Patel (8), who showed that *Ps. tumefaciens*, as well as certain closely related non-pathogenic organisms, gave negative reactions with both the Voges-Proskauer and methyl red tests.

*Thermal Death Point.* Duplicate thermal death point tests were made, employing triplicate cultures in each trial. For these trials, test tubes with walls of uniform thickness containing 5 c.c. of nutrient broth were immersed in a water bath. The water was kept in constant agitation. After it reached the desired temperature, it was kept constant for 15 minutes before inoculations of the various organisms were made. When the inoculated tubes had been held at the required temperature for 10 minutes, they were removed from the bath and placed in water at 18°C. for at least 15 minutes. A variation of 0.5°C. above or below the required temperature for a period of one minute was allowed. Trials were made at the following temperatures: 45°, 47.5°, 50°, 52.5° and 53°C.

All the strains of *Ps. tumefaciens* failed to grow in broth cultures after 10 minutes heating at 53°C., but grew after the same exposure at a tem-



perature of 52.5°. The hairyroot organisms all showed growth after exposure for 10 minutes at a temperature of 53°C. In the case of cultures 1544 and 1555 growth after three days was very slight. Of the strains of *B. radiobacter*, only culture 1455 was killed at a temperature of 53°C. This culture also failed to grow after ten minutes heating at 52.5°C., but was not killed at 50°C.

#### INFECTION EXPERIMENTS WITH CROWNGALL AND HAIRYROOT ORGANISMS

During the isolation trials, inoculations into young tomato and sugar beet plants were made to determine the pathogenicity of the organisms recovered from the apple hairyroot specimens. Reisolations were made from five of the sugar beet plants showing hairyroot and the organisms recovered were employed in the morphological and cultural studies. At the same time, infection experiments were carried on in the greenhouse and in the field, employing five apple hairyroot organisms, seven cultures of *Ps. tumefaciens* isolated from various hosts at different times, and five cultures of *B. radiobacter* previously enumerated. In these trials, inoculations were made into the tips of succulent young tomato plants, the fleshy roots of sugar beets grown in steam sterilized soil, apple seedlings three months old grown in steam sterilized soil, and into young apple trees grown in the field in non-sterilized soil. The young apple trees were three-months-old piece-root grafts. Inoculations were made by inserting the inoculum into a wound made with a flamed scalpel on the scion just above the union. After inserting the inoculum, it was pricked into the cortical tissue with a flamed needle. Inoculations were also made into the base of the young shoot below ground. This procedure was carried out with all the cultures tried. As checks, similar incisions were made with a flamed needle. The base of the shoot was similarly treated. The results of the inoculation trials are given in table 6.

As seen from the data in table 6, all the cultures of *Ps. tumefaciens* induced gall formation on tomato plants. This characteristic reaction is quite in contrast with that of the hairyroot organisms, which were unable to infect this host. No infection resulted from inoculation of tomato plants with the cultures of *B. radiobacter*. In the case of young apple trees, typical soft, spongy galls without roots or root primordia resulted from inoculation with the cultures of *Ps. tumefaciens* (Plate VA). Inoculations with the hairyroot organisms into young apple trees resulted in the development of small swellings from which hairyroots arose (Plate VA). The hairyroots were, for the most part, fleshy, but in some cases fibrous roots also arose from the overgrowth.

The results of inoculations into the crown of young apple seedlings were inconclusive. As shown in another publication (7) only a small percentage of apple seedlings inoculated in the field with *Ps. tumefaciens* isolated from raspberry developed crowngall. In the present infection studies, infection from artificial inoculation was uncertain. Cultures of *Ps. tumefaciens*, numbers 468 and R, alone induced gall formation. In these cases only one plant out of five inoculated became infected. None of the other cultures of *Ps. tumefaciens* employed induced gall formation upon apple seedlings, although all readily infected sugar beets, tomato plants, and the scion wood of three-months-old apple trees.

TABLE 6. Results of Inoculations Into Scions of Young Grafted Apple Trees, Roots of Sugar Beet, Tips of Tomato Stems and Stems of Paris Daisy With Seven Cultures of *Pseudomonas tumefaciens*, Five Cultures of Hairyroot organisms and Five Cultures of *Bacillus radiocacter*.

Cul- ture No.	Organ- ism	Pathogenic on					Symptoms produced					
		Source	Apple scion	Sugar beet	Tomato	Daisy	Apple seed- lings	Apple scions	Sugar beet	Tomato	Daisy	Apple seedlings
468	<i>Ps. tumefaciens</i>	Apple	++	++	++	—	+	Gall	Gall	Gall	—	Gall
1219		Peach	+	+	+	—	0	"	Small gall with hairyroot	"	—	0
1227		Apple	+	+	+	—	0	"	"	"	—	0
1228		Incense cedar	+	+	+	—	0	"	"	"	—	0
1236		Zygo- cactus	++	++	++	—	0	"	"	"	—	0
1303	<i>Hairyroot</i>	Hop	++	++	++	—	0	"	"	"	—	0
R		Rasp- berry	+	+	+	+	+	"	"	"	Gall	Gall
1207		Apple	+	+	+	0	+	Small swell- ing with hairyroots	Small swell- ing with hairyroots	0	Small flat- tened swell- ing with roots	Small swell- ing with hairyroots
1289		"	+	+	+	0	+	"	Gall with hairyroots	0	"	Gall
1294		"	++	++	++	0	+	"	Small swell- ing with hairyroots	0	"	Small swell- ing with hairyroots
1544	<i>B. radio- bacter</i>	"	++	++	0	—	"	"	0	"	"	
1555		"	++	++	0	—	+	"	"	0	"	
1455		—	—	0	0	—	—	—	0	0	—	
1456		—	—	0	0	—	—	—	0	0	—	
1458		—	—	0	0	—	—	—	0	0	—	
1460	<i>S</i>	—	—	0	0	—	—	—	0	0	—	
S		—	—	0	0	—	—	—	0	0	—	

In contrast to these results, all of the hairyroot organisms, induced at the point of inoculation into the apple seedling, slight swellings from which fleshy roots arose. The symptoms induced on the apple seedling were similar in appearance to those on young apple trees produced by inoculation with transfers of the same cultures and those on two-year-old nursery apple trees from which the cultures were originally isolated.

Inoculations into the crown and fleshy root of sugar beets with cultures of *Ps. tumefaciens* resulted, with one exception, in typical crown gall infection. When culture 1219 was inoculated into sugar beet, a gall, approximately 2 cm. in diameter, bearing numerous roots, developed. The gall in this case (Plate VIB1) was somewhat larger than the swellings induced upon the sugar beet by the hairyroot organisms. It is interesting to note that this culture, according to the record sent by Dr. A. J. Riker, was isolated from peach. Siegler (14) in a recent publication employs the reaction upon sugar beets as a partial means of separating the apple and peach strains of the crown gall organism. On the sugar beet the apple strain induced " . . . fleshy root-like malformations" (14, figure 4A), while the peach strain of the organism produced typical galls without roots (14, figure 4C). It is also of interest that when inoculated into young tomato plants and apple trees, culture 1219 induced typical crown galls.

The hairyroot organisms with one exception when inoculated into sugar beets, induced small swellings from which an abundance of roots arose (Plate VB). In the early stages of growth these roots were slightly swollen, but later became normal in appearance. In one series of inoculations with culture 1289, galls typical of those induced by *Ps. tumefaciens* were produced on two sugar beets, and in addition a few scattered roots arose from the gall (Plate VIB2). Root production from the galls in these plants was as abundant as in the case of the sugar beets inoculated with *Ps. tumefaciens* culture 1219 isolated from peach.

Sugar beets were also inoculated with the five cultures of *Bacillus radiobacter*. Neither galls nor hairyroot developed from these inoculations.

Paris daisy plants (*Chrysanthemum frutescens*) were inoculated with *Ps. tumefaciens* culture R and also with three cultures (1207, 1289 and 1294) of the hairyroot organism. The inoculations were made on the stems of plants about six inches in height. Typical galls developed from the inoculations with *Ps. tumefaciens*, but small, flattened swellings covered with fine, fleshy roots and root primordia developed from those inoculated with the hairyroot organism (Plate VIA). The fleshy roots were slow in growth, rarely reaching the length of one centimeter in a month's time. At this age they quickly dried and fell from the plant. The root primordia, about one millimeter in length, remained so for a period of two months, when the plants were discarded.

#### DISCUSSION

The results of the infection experiments indicate that there are three types of organisms concerned in the production of abnormalities. There are (1) those which induce gall formation, without hairyroot production, upon apple, tomato, sugar beet and Paris daisy plants, (2) those which do not infect tomato plants, but induce slight swellings with an abundant production of roots upon apple, sugar beet and Paris daisy and (3) those



which induce both galls and hairyroot upon inoculation into sugar beet. In the latter case, it is interesting to note that one organism (*Ps. tumefaciens*, culture 1219) also induced gall formation on young tomato plants, whereas with the other (hairyroot culture 1289) no infection resulted.

From the results of the inoculations it is indicated that cultures 1219 and 1289 inducing crowngall and hairyroot, respectively, upon inoculation into apple are on the border line or intermediate in their reaction with sugar beet plants. What the cause of this behavior is must remain for further study when greater numbers of cultures of *Ps. tumefaciens* and the hairyroot organisms are available for biological comparison. It appears significant that both of these organisms, as shown in table 5, are typically alkaline in their reaction on various carbohydrates. Another point of interest also shown by the morphological studies is the fact that the organisms of culture 1219 (*Ps. tumefaciens* from peach) are motile by means of polar flagella. The hairyroot organisms in culture 1289, however, are peritrichous. At first it seemed probable that the organisms with peritrichous flagella were contaminants, but subcultures from the stock culture after repeated purification in broth and dilution plates showed the same arrangement of flagella. Whether or not culture 1289 is a mixture of the crowngall and the hairyroot organisms is not known. If so, all efforts to separate the two organisms by means of repeated dilution broth and poured plate cultures have failed. While it is possible that the method used in the purification of the cultures of the hairyroot organisms was inadequate in certain cases, it appears significant that the seven cultures of *Ps. tumefaciens* and five of *B. radiobacter* were purified in the same manner and that their cultural and staining reactions as groups were quite consistent.

A comparison of the hairyroot cultures 1544 and 1555 with cultures 1207 and 1294 is also of interest from the standpoint of symptoms induced upon young apple scions and sugar beet plants and of their reactions in broths containing various carbon compounds. Cultures 1207 and 1294 were isolated from apple seedlings showing the burr-knot type of hairyroot, while cultures 1544 and 1555 were taken from small swellings at the bases of fleshy hairyroots on the scion of young nursery apple trees. The symptoms induced upon inoculation into the scion of three-months-old grafted apple trees and sugar beets appear identical, namely the production of fleshy or fibrous hairyroots from small flattened swellings. However, their reactions in broths containing various carbon compounds are directly opposed. In the cases of cultures 1207 and 1294, the reaction, while not consistent in all cases, is typically acid. The reaction with cultures 1544 and 1555, with few exceptions, is typically alkaline. The difference in the organisms is further borne out in their flagellation, as demonstrated by Casares-Gil's stain. The flagella of the organism of cultures 1207 and 1289 are peritrichous, while those of cultures 1544 and 1555 are polar. In this respect and also in their carbohydrate reactions, the latter are similar to *Ps. tumefaciens*, while the former must be considered as typical bacilli. Siegler (14) separates the organisms isolated from the woolly-knot type of crowngall on apple from those isolated from peach and daisy on the basis of the symptoms induced upon sugar beet, tomato, daisy plants, *Bryophyllum calycinum*, and apple seedlings. The organism isolated from the woolly-knot malformation on apple is designated the apple strain of the crowngall



organism. Siegler states (p. 311) that “. . . . the organism used in these experiments agrees in cultural reactions with their (Smith, Brown and Townsend) apple organism.” Attention is called to the fact that Smith et al (17) grew their apple hairyroot organism in only two sugar broths, namely sucrose and maltose. In maltose the reaction was strongly alkaline, but no record of the reaction of the organism in peptone broth containing sucrose was given. These investigators showed that the hairyroot organism was motile by means of polar flagella, and the presumption is that the organism isolated by Siegler (15) from apple is of the same type.

In a later paper Siegler (15) also gives the results of series of inoculations into apple grafts and seedlings with the peach and apple strains of the crown gall organism. The illustrations (figs. 1, 5, 7, 8) of the malformations induced by inoculations with the apple organism appear similar to those which resulted from the writers' inoculation with the hairyroot organisms (Plate VA). The type of malformation resulting from his inoculations with the peach strain on apple seedling (fig. 2) is typical of the galls induced on apple grafts by the writers using strains of *Ps. tumefaciens* isolated from apple, peach and raspberry.

It appears significant in this connection that Wright, Riker, Sagen and Banfield (20) have found that *Ps. tumefaciens* differs from the apple hairyroot organisms with respect to motility, growth characters in synthetic media, carbohydrate fermentation of nine carbon compounds, including true as well as titrable acidity, action on litmus milk, absorption of congo red, serological properties, and in host reactions.

From the results of the cultural, morphological and infection studies, it appears that two distinct types of organisms may induce the hairyroot condition when inoculated into the scions of young apple trees, stems of the Paris daisy and fleshy root of the sugar beet. In one case (cultures 1207 and 1294) the organisms are typically acid producers in carbohydrate media and are motile by means of peritrichous flagella. In the other case the organisms (cultures 1544 and 1555) are motile by means of polar flagella and with few exceptions show alkaline reaction in the 14 carbohydrate media employed. These latter organisms appear closely related to *Ps. tumefaciens*.

#### SUMMARY

In field examinations 55,668 piece-root grafted nursery apple trees in 1925, 1926 and 1927 showed 16.8 per cent, 36.1 per cent and 45.4 per cent of the trees with overgrowths. In 30,632 budded two-year-old nursery apple trees in 1925, 1926 and 1927 overgrowths were found on 6.7 per cent, 7.6 per cent and 6.5 per cent of the trees, respectively.

True crown gall, as determined by the symptoms shown in the field, occurred on an average of less than one per cent of either the grafted or budded trees. The percentage of budded trees showing hairyroot in the absence of overgrowths in the three seasons ranged from 3.4 to 0.69 and for the grafted trees from 2.7 to 0.65.

In the grafted trees 61.3 per cent of the overgrowths occurred on the tip or base of the scion lip, 14.1 per cent at the side of the union, 5.3 per cent at the tip of the stock lip, 11.7 per cent on the scion above the union, and 6.9 per cent on the stock below the union.

In isolation trials upon 183 piece-root grafted apple trees showing the hairy-knot type of malformation at the union, *Ps. tumefaciens* was recovered in one case. From 20 trees in which the overgrowths bearing fleshy roots occurred on the scion above the union, organisms non-pathogenic upon tomato, but which induced hairyroot development on the sugar beet, were recovered from seven of the specimens.

Isolation trials were made on 52 budded and 17 piece-root grafted trees showing the fibrous type of hairyroot in the absence of overgrowths. A strain of *Ps. tumefaciens* which produced gall on tomato plants was recovered from four of the budded trees.

In similar trials on 107 young budded apple trees showing the burr-knot form of hairyroot, *Ps. tumefaciens* was recovered from one specimen.

The development of clusters of fibrous roots (hairyroots) on quince cuttings has been induced in the absence of infection by *Ps. tumefaciens*. The formation of clusters of roots appears to be the natural rooting habit of the quince and is not associated with bacterial infection.

In field experiments using non-disinfected seed in unsteamed soil, overgrowths were produced only upon those apple seedlings inoculated with *Ps. tumefaciens*.

Hairyroot in the absence of overgrowths occurred independently of inoculation with *Ps. tumefaciens*, wounding or soil infestation with the crowngall organism.

In isolation trials on artificially infected apple seedlings, *Ps. tumefaciens* was recovered only from soft spongy galls lacking a bark layer and roots, but not from the hard, woody overgrowths with fleshy or fibrous roots. Trials on seedlings showing fibrous hairyroot in the absence of an overgrowth failed to yield the crowngall organism.

Isolation trials on 48 apple seedlings with the burr-knot form of hairyroot failed to yield an organism which would produce galls or hairyroots on young tomato plants. However, from three specimens, organisms were recovered which, upon inoculation into apple shoots and sugar beet plants, induced slight swellings covered with clusters of roots.

Biological studies were made of the two organisms from apple seedlings and three from the hairy-knot apple trees, which induced hairyroot upon inoculation into the crown of sugar beet plants.

All of the organisms studied were motile; the strains of *Ps. tumefaciens* by means of polar flagella; the strains of *B. radiobacter* by means of peritrichous flagella. Of the hairyroot organisms, cultures 1207, 1289 and 1294 showed peritrichous flagella, but cultures 1544 and 1555 showed polar flagella.

The strains of *B. radiobacter* are readily differentiated morphologically from both the crowngall and hairyroot organisms in the formation of capsules, the radiate arrangement of the organism in broth culture, and the distribution of the flagella.

The growth reactions of seven strains of *Ps. tumefaciens*, five strains of *B. radiobacter* and five hairyroot organisms in broths containing 14 carbon compounds were compared. In arabinose, the growth reaction of the strains of *B. radiobacter* was typically acid, while that of *Ps. tumefaciens* and the hairyroot organisms reaction was alkaline. In saccharose, *B. radiobacter* induced no change in reaction of the medium.

The growth reaction of the strains of *Ps. tumefaciens* in broths containing dextrose and salicin was acid. With the following carbon compounds—galactose, saccharose, raffinose, glycerol, arabinose, xylose, maltose, lactose, dextrin, mannite, levulose and inulin—the growth reaction was alkaline.

The hairyroot organisms may be separated into two groups on the basis of their reaction in broth cultures containing various carbon compounds, namely, (1) those producing an acid and (2) those producing an alkaline reaction in the media employed. The reactions of the alkali-producing group are similar to those of *Ps. tumefaciens*. The members of the alkali-producing group of hairyroot organisms (cultures 1544 and 1555) are motile by means of polar flagella. In the acid-producing group (cultures 1207 and 1294) the organisms are motile by means of peritrichous flagella.

Culture 1289, of the hairyroot organisms, produced an alkaline reaction with all the carbon compounds except raffinose and glycerol. In raffinose and glycerol broth there was no change in reaction in the medium. The organisms of culture 1289 showed peritrichous flagella.

All the strains of *Ps. tumefaciens* when inoculated into young tomato plants induced typical gall formation. No infection resulted from inoculations with *B. radiobacter* and the hairyroot organisms into tomato.

Inoculations with six strains of *Ps. tumefaciens* into the fleshy root of sugar beet plants resulted in typical gall formation; with four hairyroot organisms only a slight swelling with an abundance of roots was produced. Galls with a few hairyroots were developed upon sugar beet plants inoculated with one culture (1219) of *Ps. tumefaciens* isolated from peach and one of the hairyroot organisms (culture 1289).

All the hairyroot organisms induced small hard swellings with hairyroots and root primordia when inoculated into the stems of *Chrysanthemum frutescens*; *Ps. tumefaciens* induced typical large soft galls without roots.

Distinct symptoms were produced by inoculation of the scions of young apple trees with cultures of *Ps. tumefaciens* and the hairyroot organisms. In the inoculations with *Ps. tumefaciens* only galls developed, in inoculations with the hairyroot organisms small swellings with an abundance of fleshy or fibrous roots were formed.

The difference in the symptoms produced upon the scions of young apple trees, fleshy roots of sugar beet plants, and stems of *Chrysanthemum frutescens* indicate that crown gall and hairyroot may be caused by distinct organisms.

The morphological and cultural studies suggest that hairyroot symptoms upon apple scions, sugar beet and Paris daisy plants may be induced by two types of organisms. The organism may be closely related to *Ps. tumefaciens* (cultures 1544 and 1555) or unlike the crown gall pathogen in morphology or cultural reactions (cultures 1207 and 1294).



## LITERATURE CITED

1. BROWN, NELLIE A.  
1924. An apple stem-tumor not crown gall. Jour. Agric. Res. 27:695-698
2. CONN, H. J., AND G. J. HUCKER.  
1920. The use of agar slants in detecting fermentation. Jour. Bact. 5:433-434.
3. FRAZIER, W. C.  
1926. A new method for the detection of proteolysis by bacteria. (Abst.) Jour. Bact. 11:80.
4. HEDGCOCK, G. G.  
1910. Field studies of crown gall and hairy root of the apple tree. U. S. Dept. Agric. Bur. Pl. Ind. Bull. 186:1-108.
5. LÖHNIS, F., AND R. HANSEN.  
1921. Nodule bacteria of leguminous plants Jour. Agric. Res. 20:543-555.
6. MELHUS, I. E.  
1926. Crown gall of apple nursery stock. Jour. Econ. Ent. 19:356-365.
7. MUNCIE, J. H.  
1926. A study of crown gall caused by *Pseudomonas tumefaciens* on rosaceous hosts. Iowa State College Jour. Sci. 1:67-117.
8. PATEL, M. K.  
1926. An improved method of isolating *Pseudomonas tumefaciens* Sm. and Town. Phytopath. 16:577.
9. ————  
1929. Biological studies of *Pseudomonas tumefaciens* Sm. and Town. and fifteen related non-pathogenic organisms. Iowa State College Jour. Sci. 3:271-298.
10. RIKER, A. J., AND G. W. KEITT.  
1926. Studies of crown gall and wound overgrowth on apple nursery stock. Phytopath. 16:765-808.
11. ————  
1925. A report of progress on studies of crown gall in relation to nursery stock. Science 62:184-185.
12. ————  
1925. Second report of progress on studies of crown gall in relation to nursery stock. Phytopath. 15:805-806.
13. ————, W. M. BANFIELD AND G. W. KEITT.  
1929. The relation of certain bacteria to the development of roots. (Abst.) Phytopath. 19:107.
14. SIEGLER, E. A.  
1928. Studies on the etiology of apple crown gall. Jour. Agric. Res., 37:301-313.
15. ————  
1929. The woolly-knot type of crown gall. Jour. Agric. Res., 39:427-450.
16. SMITH, E. F., AND C. O. TOWNSEND.  
1907. A plant tumor of bacterial origin. Science n. s. 25:671-673.
17. ————, NELLIE A. BROWN AND C. O. TOWNSEND.  
1911. Crown-gall of plants: its cause and remedy. U. S. Dept. Agric. Bur. Pl. Ind. Bull. 213:1-200.
18. STEWART, F. C., F. M. ROLFS AND F. H. HALL.  
1900. A fruit disease survey of Western New York in 1900. N. Y. (Geneva) Agric. Exp. Sta. Bull. 191:300-301.
19. SWINGLE, C. F.  
1925. Burr-knot of apple trees. Its relation to crown gall and to propagation. Jour. Heredity 16:313-320.
20. VOGES, O., AND B. PROSKAUER.  
1898. Beiträge zur Ernährungsphysiologie und zur Differential-diagnose der Bakterien der Hämorrhagischen Septicämie. Ztschr. Hyg. u. Infektionskrank. 28:20-32.
21. WRIGHT, W. H., A. J. RIKER, H. E. SAGEN AND W. M. BANFIELD.  
1929. Studies on the bacteriological differentiation of the crown gall and hairyroot types of bacteria. (Abst.) Phytopath. 19:97-98.



## PLATE I.

- A—Two-year-old grafted apple tree, variety Wolf River, showing hairy-knot (woolly-knot) condition. These are typical of the trees with hairy-knot at the union, from which isolations were made.
- B—Two-year-old Sweet Russet apple trees with fleshy hairyroots developed from the scion. Photographed after making isolations. From each specimen an organism was recovered which induced hairyroot formation when inoculated into sugar beet plants. (See Plate VB.)

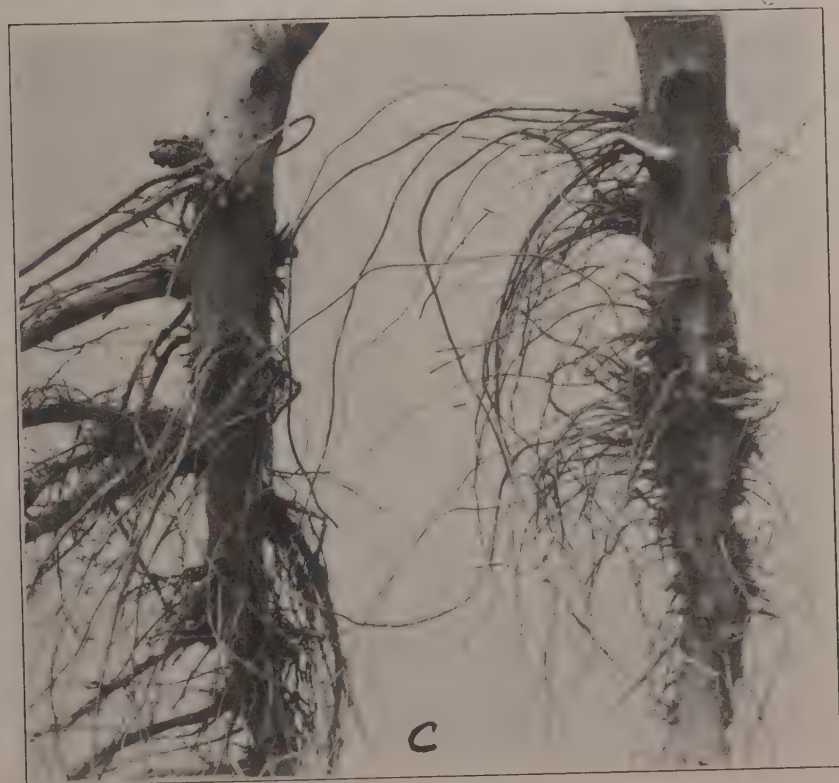
PLATE I.



## PLATE II.

- A—A two-year-old Delicious apple tree budded upon French seedling showing the fibrous type of hairyroot. This type of hairyroot is characteristic of the condition found on nursery trees grown in sandy soil.
- B—A two-year-old Jonathan budded upon an Italian seedling showing the burr knot type of hairyroot. Note the clusters of aborted fleshy roots arising from the swellings on the stock. Natural size.
- C Two trees of the variety Yellow Transparent, budded upon Kansas grown seedlings, showing the burr-knot type of hairyroot.

PLATE II.





## PLATE III.

- A—Trees of the varieties Wealthy, Jonathan and Delicious budded on Italian seedlings, showing the burr-knot type of hairyroot. In these specimens aborted fleshy roots arose in clusters from small flattened swellings along the main root.
- B—Two Orango quince trees showing the development of clusters of thickened fibrous roots from the stock. These trees were discarded by the nurseryman as affected with bacterial hairyroot.

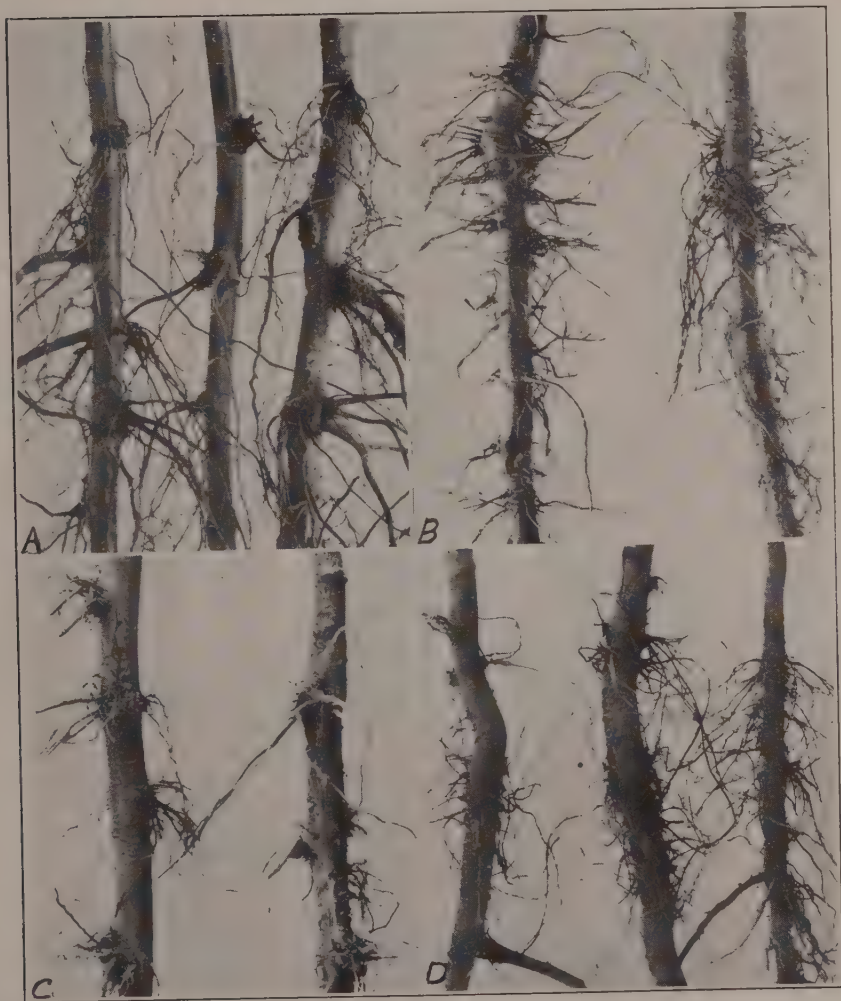
PLATE III.



## PLATE IV.

- A—Imported rooted quince cuttings used for budding.
- B—Kansas grown apple seedlings showing the burr-knot type of hairyroot. From the bases of the clusters of roots, organisms were obtained which induced hairyroot formation on sugar beet plants and young apple trees.
- C—Development of fleshy roots from two of the above quince specimens after storage in moist sphagnum for four weeks.
- D—Kansas grown apple seedlings showing the development of fibrous roots in clusters. Such seedlings are often discarded by nurserymen as affected with hairyroot.

PLATE IV.

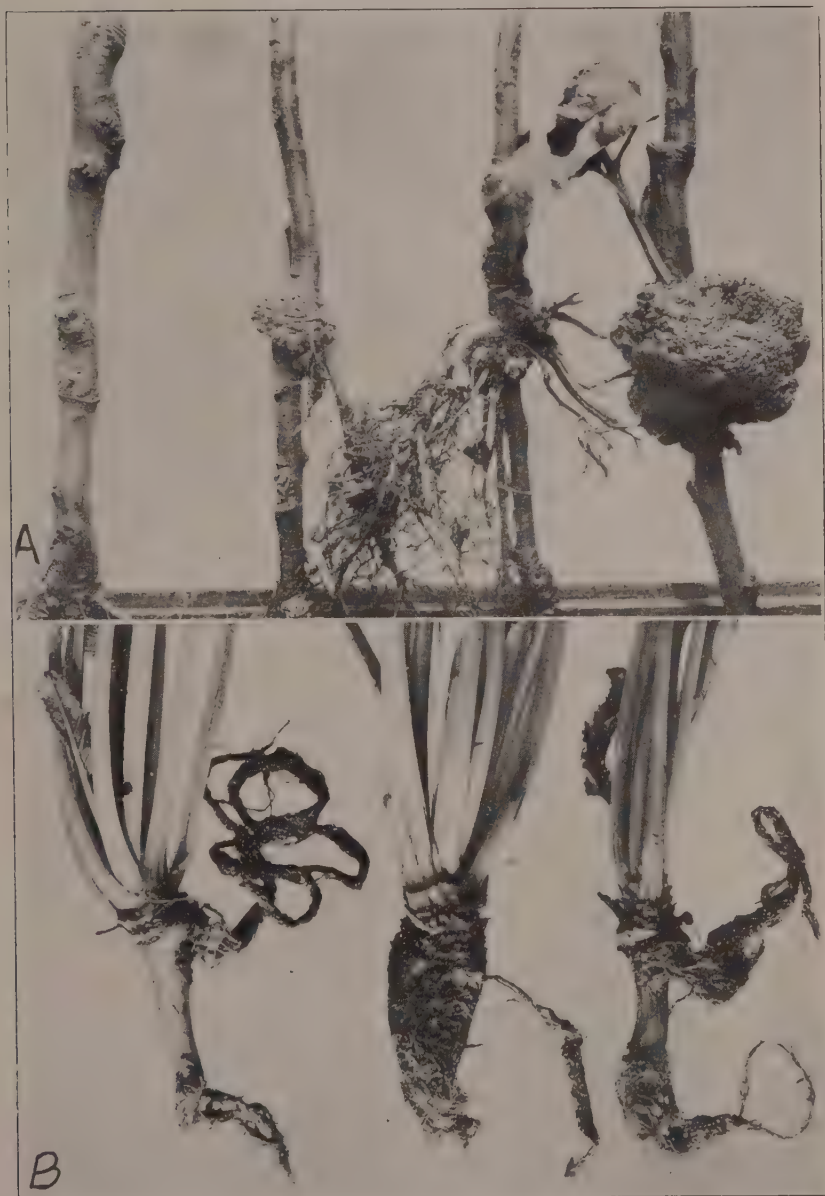




## PLATE V.

- A—Young apple grafts showing the results of inoculation with hairyroot and crown gall organisms. Left to right: check, plants inoculated with hairyroot organism cultures 1207 and 1289 and the raspberry strain of *Ps. tumefaciens*. Note fleshy roots arising from the swellings induced by the hairyroot organisms.
- B—Three sugar beets inoculated with hairyroot organism cultures 1544, 1294 and 1555. Note the development of roots from slight swellings at the point of inoculation.

PLATE V.



## PLATE VI.

A—*Chrysanthemum frutescens* showing the results of inoculation with hairyroot and crown gall organisms. Right to left: check, hairyroot cultures 1544 and 1207 and apple strain of *Ps. tumefaciens*. The hairyroot cultures induced only slight swellings from which fleshy roots and root primordia developed. The fleshy roots after five months became dessicated and were broken off.

B1—Sugar beet inoculated with hairyroot culture 1289 showing the development of a large gall with an abundance of roots. This type of infection appears to be intermediate between that caused by *Ps. tumefaciens* and the hairyroot organisms on this host.

B2—Gall and hairyroot from inoculation with *Ps. tumefaciens* culture 1219 isolated from peach.

Both plants grown in steam sterilized soil.

PLATE VI.





## PLATE VII.

A—*Pseudomonas tumefaciens* culture R, isolated from crown gall on raspberry.

B—Apple hairyroot organism, culture number 1289.

C—Apple hairyroot organism, culture number 1207.

D—*Bacillus radiobacter*, culture 1455.

E—*Bacillus radiobacter*, culture S.

F—Apple hairyroot organism, culture 1294.

PLATE VII.





## CROWNGALL OF RUMEX CRISPUS L. AND RHEUM RHAPONTICUM L.

J. H. MUNCIE\*

*From the Department of Botany, Iowa State College*

Accepted for publication October 15, 1929

For the most part, the known hosts of the crown gall pathogen, *Pseudomonas tumefaciens* Sm. and Town., are plants of economic importance. Because of the wide variety of such plants attacked, as shown by Smith (3, p. 430), little search has been made for the crown gall disease on other hosts. Evidence has been obtained (2) showing that the common weed, curly dock (*Rumex crispus* L.) and rhubarb (*Rheum rhaponticum* L.) may also harbor the crown gall organism. The occurrence of crown gall on rhubarb is of interest because it represents an unusual type of infection; that of the fleshy underground stem or rhizome. These two new hosts of *Pseudomonas tumefaciens* are of further interest because of the high acidity of the plant juices.

### PATHOGENICITY OF PSEUDOMONAS TUMEFACIENS ON RUMEX CRISPUS AND RHEUM RHAPONTICUM

A plant of *Rumex crispus* showing a large tumorous overgrowth on the tap root, just below the crown, was handed to the writer by Dr. O. H. Elmer, June 24, 1926. The specimen was found in a Minnesota raspberry plantation growing between the rows of plants. Although no record was obtained as to the proximity of the specimen of *Rumex* to galled raspberries, crown gall was present on many of the raspberry plants. The gall on *R. crispus* (Plate I) was typical of those occurring on raspberry plants at the crown or upon the fleshy root of the sugar beet. The gall measured 5 x 5 cm., with an elevation of 3.5 cm. The gall originated in a wound on the tap root just below the crown. The occurrence of a large tumorous overgrowth on *R. crispus* was of unusual interest in view of the statement by Smith and Quirk (4) that "... it is still believed that crown gall tumors on such plants (*Rumex* sp. *Begonia* sp. etc.) are non-existent in nature."

Isolations, therefore, were made from the gall and *Pseudomonas tumefaciens* recovered in almost pure cultures. In the greenhouse, inoculations were made into 12 young tomato plants with streaks from the original plates, and typical galls resulted in every case. Reisolations from the tomato galls were made, and the pathogen recovered in almost pure culture.

---

\*The writer is indebted to Dr. I. E. Melhus for critical reading of the manuscript, and suggestions during the course of the investigation. These studies have been carried out at Iowa State College in connection with the crown gall project in which the Crop Protection Institute, Iowa State College and the United States Department of Agriculture, Office of Horticultural Crops and Diseases, are cooperating.



Further inoculations were made, September 18, 1926, into 15 plants of *Rumex crispus* growing naturally in an alfalfa field. The tap roots of these plants at the crown were approximately 2 cm. in diameter.

The finding of the galled *Rumex* plant in a raspberry plantation where crown gall was present, suggested that the raspberry strain of *Ps. tumefaciens* was able to infect this weed.

For the inoculation trials, cultures from two sources were employed: (1) the pathogen isolated in 1924 from a red raspberry gall, and (2) the pathogen originally isolated from the *Rumex* gall. Six *Rumex* plants were inoculated with the *Rumex* strain of the organism and five plants developed small galls (Plate II, fig. 1). Of the nine *Rumex* plants inoculated with the raspberry strain of *Ps. tumefaciens*, seven showed typical infection. Re-isolations were made from galls from each series of inoculations and used to infect young tomato plants.

Although the source of infection on the original plant of *Rumex crispus* is not known, the results of the inoculations show that the raspberry strain of *Pseudomonas tumefaciens* is capable of producing infection on this host. The prevalence of *Rumex crispus* in nursery fields, and the ease with which it may be infected suggest the possibility of this host carrying over the disease from year to year in fields planted to non-susceptible crops. It is of interest, also, that so far as the writer is aware, *Rumex crispus* is the first naturally infected weed host reported for the crown gall pathogen.

A second unreported host for *Pseudomonas tumefaciens* was found March 31, 1929, when three rhizomes of rhubarb (*Rheum rhaponticum*) showing small crown galls (Plate II, fig. 2) were found at an Iowa nursery. These infected rhizomes had been removed for sorting from the storage cellar. They had been dug during the fall from a field from which young Bolleana poplar trees, showing a high percentage of crown gall, were taken two years previously. *Pseudomonas tumefaciens* was isolated from the gall, and sub-cultures were used to infect four young sugar beet plants, four tomato plants, and the rhizomes of two rhubarb plants.

The finding of *Rheum rhaponticum* as a host for the crown gall pathogen is of considerable interest because of the high acid content of the plant juice and also because this represents an unusual type of infection; the fleshy underground stem or rhizome.

#### RELATION OF HOST ACIDITY TO INFECTION

The results of the isolation and inoculation trials with *Rumex crispus* and *Rheum rhaponticum* having shown them quite susceptible to crown gall infection, directed attention to the relation between host acidity and infection.

Smith and Quirk (4) in their studies on immune or semi-immune plants were unable to infect stems of *Begonia coccinea* Hook. hort. var. *lucerna* with *Pseudomonas tumefaciens*. Hydrogen ion determinations on extracted plant juice showed a pH range of 0.90 to 1.36 for the leaves, 1.33 to 2.23 for the top of the shoots and 3.30 to 3.42 for the base of the shoot. Similar electrometric determinations were made on juice extracted from leaves of three other species of begonia, *Rumex crispus* and *R. obtusifolius* L. In all cases the acidity of the juice (pH 3.53 to 3.67) was well beyond the acid tolerance of *Pseudomonas tumefaciens* grown in peptonized bouil-

lon (pH 5.8). No attempt was made to inoculate either species of *Rumex*, although stems of *Begonia phyllomoniaca* Mart. (pH 1.52 to 1.94) were artificially infected with the crown gall pathogen.

The development of large galls on the root of *Rumex crispus* both in nature and from artificial infection, suggested that either the juice of the root was less acid than that of the leaves, or that infection was due to the ability of the organism to adjust the reaction of the medium to its own requirements. Therefore, hydrogen ion determinations, using the colorimetric method, were made on juice extracted from the leaves and roots of healthy and galled plants of *Rumex crispus*, and also from the gall tissue on the root. Triplicate trials using six plants showed the average pH values of the juice from leaves to be 4.8, from healthy roots 5.2, and from the gall tissue of the root, 5.9.

Here it is of interest to note that Israelsky (1) found that the juice of crown gall tissue of sugar beet and rape plants showed a greater alkalinity than the uninfected portions of the same plant.

Similar trials were also made with rhubarb plants. Healthy rhubarb rhizomes and those showing crown gall infection were transplanted to pots in the green house, and when the leaf stalks had grown to a height of 12 inches, hydrogen ion determinations were made on juice extracted from healthy shoots and rhizomes and from the gall tissue on the rhizomes. Triplicate tests, employing the colorimetric method, showed the plant parts to have the following acidity: shoot, pH 3.7; rhizome, pH 5.5; and gall tissue, pH 5.7.

The results of the hydrogen ion determinations on *Rumex crispus* and *Rheum rhaponticum* show that there is a wide difference between the acidity of the juice of the stems and leaves and that of the roots and rhizomes. Although the hydrogen ion concentration of the juice of the leaves and shoots was found to be higher than the acid toleration (pH 5.8) of *Pseudomonas tumefaciens* in broth culture, that of the roots and rhizomes was appreciably lower, namely 5.2 and 5.5, respectively. The ease with which infection was induced by inoculation of the roots of *Rumex crispus* and the rhizomes of *Rheum rhaponticum* indicates that *Pseudomonas tumefaciens* may establish itself in a medium of relatively high acidity, and adjust the reaction of the medium to suit the growth requirements of the organism.

#### LITERATURE CITED

1. ISRAILSKY, W. P.  
1927. Bakteriophage und Pflanzenkrebs. Centralbl. Bakt. II Abt. 71:302-313.
2. MUNCIE, J. H.  
1928. *Rumex crispus*, a weed host of *Pseudomonas tumefaciens*. (Abst.) Phytopath. 18:130.
3. SMITH, E. F.  
1920. Bacterial diseases of plants. Philadelphia and London. 688 pp.
4. SMITH, E. F. AND AGNES J. QUIRK  
1926. A begonia immune to crown gall: with observations on other immune or semi-immune plants. Phytopath. 16:491-508.

## PLATE I

*Rumex crispus* L. showing natural infection by *Pseudomonas tumefaciens*.

PLATE I





## PLATE II

Fig. 1. Plants of *Rumex crispus* L. showing artificial infection by *Pseudomonas tumefaciens*. At left, plant inoculated with the raspberry strain and at right, inoculated with the *Rumex* strain of the crown gall pathogen.

Fig. 2. Natural infection of *Rheum rhaponticum* L. by *Pseudomonas tumefaciens*. The older portion of the crown galls (shown at either side of the specimen) have sloughed off, leaving only a small overgrowth.

PLATE II



Fig. 1

Fig. 2



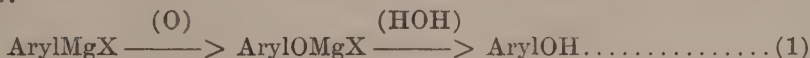
# THE OXIDATION OF PHENYLMAGNESIUM BROMIDE IN THE PRESENCE OF TETRAETHYL LEAD

HENRY GILMAN AND J. A. LEERMAKERS

*From the Chemical Laboratory, Iowa State College*

Accepted for publication Nov. 26, 1929

When arylmagnesium halides are oxidized, phenols are formed as follows:



This reaction is one of considerable importance inasmuch as an unusually wide variety of phenols can be prepared in this manner. Unfortunately, however, the yields of hydroxyl compounds formed by such oxidation are unsatisfactory<sup>1</sup>. Gilman and Wood<sup>2</sup> have shown that the poor yields are due primarily to the oxidation of the ether (used as a solvent for the RMgX compounds) to a peroxide. The formation of this peroxide of ether results in by-products, particularly secondary alcohols.

Accordingly, one way of improving the yield of phenolic compound in this reaction is to inhibit or reduce the oxidation of ether peroxide<sup>3</sup>. Recent studies<sup>4</sup> on the causes which under-lie the knocking effect in internal combustion engines, have suggested a possible solution to this problem. It has been found that a number of compounds inhibit the knock of some fuels, possibly by the prevention or reduction of peroxide formation of the fuels. By analogy, it seemed of promise to carry out the oxidation of arylmagnesium halides in the presence of an inhibitor of peroxide formation in the hope of avoiding the side reactions that are due to the ether peroxide. With this as a basis, 0.2 mole of phenylmagnesium bromide was oxidized in a customary manner in the presence of 0.75 c.c. of tetraethyl lead (the standard anti-knock compound). The yield of phenol, however, was no different in the presence of this anti-catalyst than the yield obtained in the absence of catalysts<sup>5</sup>. In a related experiment Mr. C. D. Schmidt showed that 0.5 g. of lead dioxide was without effect on the yield of phenol by the oxidation of 0.1 mole of phenylmagnesium bromide.

<sup>1</sup>This is in rather sharp contrast with the corresponding *alkyl*magnesium halides which, on oxidation, give satisfactory yields of the corresponding alcohols.

<sup>2</sup>Gilman and Wood, *J. Am. Chem. Soc.*, **48**, 806 (1926).

<sup>3</sup>There are other possible methods such as the use of ethers which are resistant to oxygen (for example, di-*tert.* butyl ether, and the oxidation of RMgX compounds in media or solvents other than diethyl ether. At present, however, no solvent is as satisfactory as diethyl ether for effecting Grignard reactions.

<sup>4</sup>One of the best and most recent accounts of such studies is that by Egloff, Schaad and Lowry Jr., *Ind. Eng. Chem.* **21**, 785 (1929). This article contains an excellent bibliography on anti-knock studies and the oxidation mechanisms of paraffin hydrocarbons.

<sup>5</sup>Possibly the desired result can be obtained by the use of a catalyst to accelerate the oxidation of phenylmagnesium bromide, to an extent greater than the formation of ether peroxide. Studies by Edith L. St. John, now in progress, show that *p*-dichlorobenzene accelerates some RMgX reactions.





## PHOSPHORUS ASSIMILATION BY CERTAIN SOIL MOLDS\*

P. E. BROWN AND F. B. SMITH

*From the Laboratory of Soil Chemistry and Bacteriology, Iowa State College*

Accepted for publication January 13, 1930

It has long been recognized that a rather definite relationship exists between the crop producing power of soils and their content of available phosphorus. The factors influencing the availability of the insoluble phosphates normally occurring in the soil, have been the subject of many investigations. It has been found that the microorganisms present in the soil are the chief agents bringing about the solubility of the phosphorus compounds. Some studies have dealt with the whole question of the phosphorus cycle in soils, while others have considered practical problems involved in bringing about a greater production of available phosphorus in field soils. Still other work has centered around the reinforcing of stable manure with phosphates, and the composting of rock phosphate with easily decomposable organic matter or with flowers of sulfur with or without inoculation. Some studies have been carried out to determine the effect of individual organisms on the availability of phosphorus compounds.

In connection with a number of these investigations, it has been noted that the action of soil microorganisms does not always result in an increase in the amount of soluble phosphate, but actually may bring about a decrease. In other words, available phosphorus in soils may be assimilated by certain organisms and utilized by them for food, such assimilation occasionally reaching an amount which places the organisms in actual competition with the higher plants. The process of phosphorus assimilation by microorganisms, then, is of interest technically and may prove of practical significance also.

De Grazia and Cerza (2) found that *Aspergillus niger*, *Penicillium glaucum* and *P. brevicaulis* were active in rendering tricalcium phosphate soluble in culture media. They noted, however, that, in the field, a part of the phosphorus dissolved by the action of the microorganisms is utilized by them in their growth.

Dox (3) reported that a variety of phosphorus-containing substances may be utilized by *Aspergillus niger*, but the trivalent phosphates are not assimilated.

Egorov (6) noted a reduction in the amount of phosphoric acid as inorganic phosphates, in manure in which a moisture content of about 43 per cent was maintained and which was kept in closed flasks at 35-37°C. for two months and subjected to the action of a current of air.

Sewerin (9) studied the effect of bacteria on the solubility of tricalcium phosphate and found the amount of water-soluble phosphorus materially lowered. It was concluded that bacteria utilized some of the soluble phosphates and chemical reactions also occurred, transforming them into

---

\*The molds used were isolated by Dr. E. V. Abbott, and are described in: Gilman, J. C. and E. V. Abbott. 1927. A Summary of the Soil Fungi IOWA STATE COLLEGE JOURNAL OF SCIENCE, 1:225-343.

less soluble forms. In later work (10) the same author noted a decrease in the soluble phosphoric acid in soils which were first sterilized and then inoculated with soil bacteria. He also found (11) that a soil not sterilized, lost 12.6 per cent of the soluble phosphorus; when sterilized and inoculated with *Bacillus fluorescens liquefaciens* there was a decrease of 5.8 per cent, while when sterilized and inoculated with *Azotobacter*, *Bacillus mesentericus vulgatus* and *B. mycoides*, increases in soluble phosphorus occurred.

Stoklasa (12) called the assimilation of phosphates by bacteria "biological absorption," and reported 98 per cent of  $P_2O_5$  absorbed by an inoculated soil, compared with 66 per cent in an uninoculated soil. He concluded that the "biological absorptive capacity" of a soil is closely related to its productivity.

Dowell (4) found no increase in the water-soluble phosphate of a compost of mixed manure and rock phosphate after fermenting for a year.

Tottingham and Hoffman (13) reported decreases in water-soluble phosphorus in manure, and in mixtures of manure and rock phosphate when fermented for four or six months. In the mixture, the losses amounted to more than one-half the soluble phosphorus originally present. Adding chloroform or formalin greatly lessened the losses, indicating that the action of bacteria is mainly responsible.

Dushechkin (5) concluded that in addition to the physico-chemical absorption of phosphoric acid in the soil, there is also a biological absorption which is influenced by the phosphorus present, the starch content of the soil, the water content, the presence of sodium nitrate, the light and the organisms present.

Sackett, Patten and Brown (8) carried out experiments which showed increases in available phosphorus from the action of certain bacteria, but in other cases decreases were noted. Assimilation undoubtedly had occurred in some instances.

Bazarevski (1) found that the addition of carbohydrates to the soil brought about an increase in the number of microorganisms present, and a decrease in the phosphoric acid soluble in two per cent acetic acid.

It has been concluded that phosphate assimilation by soil microorganisms may take place to a considerable extent, especially when a large amount of energy-bearing material is added to the soil without a corresponding addition of soluble phosphate. The microorganisms are stimulated to greater action and hence greater assimilation of phosphates occurs, sometimes increasing to an undesirable extent. The development of the process has been found to be particularly vigorous in manure and in composts of manure and rock phosphate.

#### EXPERIMENTAL

The purpose of this work was to study the effect of some common soil molds on the amount of available, or water-soluble phosphorus in soil and in solution cultures.

Pure cultures of the organisms were inoculated into the culture medium and determinations of water-soluble phosphorus were made at intervals. Two series were run in solution cultures, the incubation periods being 45 and 71 days. One series was run in soil cultures with an incubation period of 60 days.

## SERIES I

The medium used in the solution cultures consisted of

NaNO <sub>3</sub> .....	20.0 gms.
KCl .....	10.0 gms.
MgSO <sub>4</sub> .....	5.0 gms.
FeSO <sub>4</sub> .....	1.0 gm.
Sucrose .....	300.0 gms.
Water .....	1000.0 c.c.

One hundred c.c. portions of this solution were diluted to one liter and sterilized at 15 pounds pressure for 15 minutes. To 200 c.c. portions of this medium in one liter Erlenmeyer flasks, 5 grams of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> were added and the solutions were sterilized in the autoclave for 10 minutes at 10 pounds pressure. Duplicate flasks were inoculated with a one c.c. suspension of spores of the various organisms and the cultures were incubated at 25°-26°C. for 45 days. A second set of cultures made up in the same way was allowed to incubate 71 days. After incubation, the cultures were filtered through large Buechner funnels and washed with 300 c.c. of distilled water. The filtrate was made up to 500 c.c. and total soluble phosphorus determined by the colorimetric method (7).

The results, secured when the cultures were incubated 45 days, are given in table 1. *Tetracoccosporium paxianum* increased the amount of water soluble phosphorus 0.092 mgm., but *Penicillium pinophilum* and *Scolecobasidium constrictum* reduced the amount of soluble phosphorus by 0.672 and 0.157 mgm., respectively. These two organisms evidently are able to assimilate phosphorus in considerable amounts.

*Tetracoccosporium paxianum* decreased the hydrogen ion concentration from pH 4.91 to pH 5.85. *Penicillium pinophilum* increased the acidity from pH 4.91 to pH 4.57, while *Scolecobasidium constrictum* had little effect on the reaction of the medium.

TABLE 1. *Phosphates Assimilated from Solution by Cultures of Molds in 45 Days.*

Organism	Reaction of medium pH	Mgm. PO <sub>4</sub> per culture
Control .....	4.91	1.212
<i>Tetracoccosporium paxianum</i> .....	5.85	1.304
<i>Penicillium pinophilum</i> .....	4.57	0.540
<i>Scolecobasidium constrictum</i> .....	4.93	1.055

*Tetracoccosporium paxianum* apparently brought about a large production of soluble phosphate, but a large amount of the phosphate was assimilated, leaving the medium less acid in reaction. *Penicillium pinophilum* assimilated a considerable amount of the soluble phosphorus, but the reaction was more acid after 45 days. The third organism assimilated soluble phosphorus with little change in the reaction of the medium.

The results secured in 71 days are given in table 2. All the cultures showed less water-soluble phosphorus than the control and evidently are active in phosphorus assimilation.



TABLE 2. *Phosphates Assimilated from Solution by Cultures of Molds in 71 Days.*

Organism	Reaction of medium pH	Mgm. PO <sub>4</sub>
Control .....	4.95	3.332
<i>Aspergillus flavipes</i> .....	6.80	3.079
<i>Aspergillus wentii</i> .....	6.98	2.640
<i>Zygorhynchus vuilleminii</i> .....	5.00	2.700
<i>Aspergillus minutus</i> .....	4.90	2.780
<i>Trichoderma lignorum</i> .....	4.96	2.860
<i>Penicillium vinaceum</i> .....	4.30	2.780
<i>Spicaria violacea</i> .....	6.97	2.560
<i>Penicillium rugulosum</i> .....	4.51	2.635
<i>Gliocladium deliquescens</i> .....	4.56	2.500

*Gliocladium deliquescens* assimilated the largest amount of phosphorus, while *Aspergillus flavipes* showed the least effect. The latter organism, however, changed the reaction of the medium from pH 4.95 to 6.80, while the former brought about an increase in acidity. *Aspergillus wentii* and *Spicaria violacea* changed the pH from 4.95 to 6.98 and 6.97, respectively, but both showed high phosphorus assimilating power. Three of the cultures showed little effect on the reaction, three produced acidity and three alkalinity, but all assimilated phosphorus in appreciable amounts. No definite relation between the change of reaction and the amount of phosphorus assimilated can be seen.

## SERIES II

Webster loam was used in the soil cultures. Equivalents of 100 grams of dry soil were made up to 50 per cent of the saturation capacity, with distilled water in one liter flasks and sterilized in the autoclave at 15 pounds pressure for three hours. No organisms developed on plates poured on Waksman's synthetic acid medium from these soils. Duplicate cultures were inoculated with one c.c. suspensions of spores of the various organisms and incubated at 25°-26°C. for 60 days. After incubation, 500 c.c. of distilled water were added to each culture and the flask shaken at intervals of 20 minutes for two hours. The extract was then filtered in a Buechner funnel with a slight suction and the water-soluble phosphorus determined on an aliquot by the colorimetric method. The total water-soluble phosphorus was determined in eight successive extracts of 500 c.c. each, and 96 per cent of this total was secured in the first 500 c.c. of extract. The results secured are given in table 3.

TABLE 3. *Phosphates Assimilated from Soil by Molds in 60 Days.*

Organism	Mgm. PO <sub>4</sub> in 100 gms. dry soil
Control .....	0.109
<i>Aspergillus terreus</i> .....	0.066
<i>Aspergillus sulphureus</i> .....	0.025
<i>Aspergillus luchuensis</i> .....	0.094
<i>Aspergillus minutus</i> .....	0.034
<i>Penicillium vinaceum</i> .....	0.111
<i>Penicillium chrysogenum</i> .....	0.100
<i>Penicillium humicola</i> .....	0.060
<i>Penicillium pinophilum</i> .....	0.070

All of the cultures except that of *Penicillium vinaceum* showed an assimilation of phosphorus. *Aspergillus sulphureus* gave the greatest assimilation and *Aspergillus minutus* also showed a high assimilating power. *Penicillium chrysogenum* showed only a slight assimilation.

## SUMMARY

Twenty cultures of molds were incubated in solution and in soil cultures for 45, 71 or 60 days. With two exceptions, the organisms showed a definite ability to assimilate phosphorus. *Tetracoccusporium paxianum* in solution culture gave an increase in water-soluble phosphorus, all the others showing a decrease or a phosphate assimilation. Possibly that organism also assimilated soluble phosphorus, but the effect in producing soluble phosphorus may have been unusually great, exceeding its assimilating power. One organism, *Penicillium vinaceum*, had no assimilating power in the soil culture test.

The ability to assimilate soluble phosphorus seems rather common to soil molds, if the cultures tested here can be considered representative. No definite relationship between assimilating power and change of reaction in culture solutions could be shown.

## LITERATURE CITED

1. BAZAREVSKI, S.  
1916. On the question of mobilization of phosphoric acid in soil by the agency of microorganisms. (Russian) Moskau. Ref. Waksman's Principles of Soil Microbiology, Williams and Wilkins, Baltimore. p. 653.
2. DE GRAZIA, S. AND V. CERZA.  
1907. On the intervention of microorganisms in the utilization of insoluble phosphates of the soil by plants. Arch. Farmacol. Sper. e Sci. Aff. 6, No. 1:6. Exp. Sta. Rec. 18:920 (Abst.).
3. DOX, A. W.  
1911. The phosphorus assimilation of *Aspergillus niger*. Jour. Biol. Chem. 10:77.
4. DOWELL, M. S.  
1908. Is the phosphoric acid of floats made soluble by rotting manure? Pa. Agric. Exp. Sta. Rpt. 1907-08:175-178.
5. DUSHECHKIN, A. J.  
1911. The biological absorption of phosphoric acid in the soil. Russ. Jour. Expt. Landw. 12 (5):650-668. Exp. Sta. Rec. 27:216 (Abst.).
6. EGOROV, M. A.  
1925. The different conditions of decomposition of manure and its phosphoric acid. Fosfornia Kislota Navoza pri Razlichnykh Usloviakh ego Razlozheniia (Kharkof): Gosud. Izdatel. Ukrain. pp. 130. English Abst. p. 105. Exp. Sta. Rec. 57:316 (Abst.).
7. PARKER, F. W. AND J. F. FUDGE.  
1927. Soil phosphorus studies. I. The colorimetric determination of organic and inorganic phosphorus in soil extracts and the soil solution. Soil Science 24 (2):109.
8. SACKETT, W. G., A. J. PATTEN AND C. W. BROWN.  
1908. The solvent action of soil bacteria upon the insoluble phosphates of raw bone meal and natural raw rock phosphate. Mich. Agric. Exp. Sta. Sp. Bull. 43.

9. SEWERIN, S. A.  
1910. The mobilization of the phosphoric acid in soil under the influence of bacterial activity. *Centbl. Bakt. etc. II Abt.* **28** (22/24):561-580.
10. \_\_\_\_\_  
1912. The mobilization of the phosphoric acid in soil under the influence of bacterial activity II. *Centbl. Bakt. etc. II. Abt.* **32** (20/25):498-520.
11. \_\_\_\_\_  
1914. The mobilization of soil phosphoric acid under the influence of bacteria IV. *Vienstnik Bakt. Agron. Sta. V. K. Ferrein. No. 21:53-83. Exp. Sta. Rec.* **36:515** (Abst.).
12. STOKLASA, J.  
1911. Biochemische Kreislauf des Phosphat-Ions im Boden. *Centbl. Bakt. etc. II. Abt.* **29** (15/19):385-515.
13. TOTTINGHAM, W. A. AND C. HOFFMAN.  
1913. Nature of the changes in the solubility and availability of phosphorus in fermenting manures. *Wis. Agric. Exp. Sta. Res. Bull.* **29**.

## THE BIOLOGICAL ESTIMATION OF GLUCOSE

### I. A STUDY OF FACTORS INFLUENCING CHANGES IN H-ION CONCENTRATION OF MEDIA\*

AMY LEVESCONTE WITH J. H. BUCHANAN AND MAX LEVINE

*From the Department of Chemistry, Iowa State College*

Accepted for publication January 15, 1930

An accurate quantitative chemical determination of mixtures of sugars is difficult. The properties of all sugars are similar, and as a consequence, reagents that will react with one sugar in a mixture often react to some extent with the other sugars. These reactions result in serious errors in the determination. It is especially difficult to obtain satisfactory results for a sugar present in low concentration in the presence of others in higher concentration.

It has been demonstrated many times that the methods of analyzing sugar mixtures, based on the action of enzymes, yeasts, or bacteria are dependable. Pasteur (8) studied the levo- and dextro-isomers of tartaric acid with the aid of microorganisms. Pottevin (9), Bourquelot (1), Bourquelot et Herissey (2), and others, have made use of enzyme action to detect glucosides and to determine their concentration in plant juices. Davis (4) has outlined a method for the determination of various sugars in a mixture by the selective decomposition of these sugars by enzymes. Castellani and Taylor (3) and Kendall (6) were successful in identifying sugars in mixtures by the action of microorganisms. By making a measure of hydrogen ion concentration changes in dilute sugar solutions, inoculated with certain strains of bacteria, Kendall and Yoshida (7) demonstrated the possibility of estimating sugars in small concentrations by this method.

Since biological agents are known to be more selective in their action than chemical reagents, and since many microorganisms bring about definite changes in pH of sugar solutions, it seemed possible to determine glucose and other carbohydrates in low concentration by the use of definite organisms, using change in pH as a measure of the amount of carbohydrate present. This study has been restricted to the estimation of glucose, using this sugar in varying concentrations, in pure solution, and in the presence of sucrose.

In these experiments a method was outlined using synthetic media consisting of dilute solutions of  $(\text{NH}_4)_2\text{HPO}_4$  and KCl with glucose in different concentrations. After sterilization in the autoclave and inoculation with the organism, the tubes of media were incubated in a thermostat at 30°C. At definite intervals of time the pH was determined with a potentiometer, using a quinhydrone electrode. From these observations conclusions are drawn, first with regard to the factors influencing change of pH

---

\*A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Iowa State College.



of media, and second with regard to the application of these results to the development of a practicable biochemical method of analyzing sugar mixtures.

#### METHOD OF PROCEDURE

The organism used, a strain of *Aerobacter levans*, was particularly suitable for this work because of the fact that it produces acid from glucose in an otherwise inorganic synthetic medium. Being a facultative aerobe, it will grow either under aerobic conditions with the production of acid, followed by a decomposition of the acid formed, or under anaerobic conditions with the simple production of acid.

The culture was maintained on dextrose agar slants. Before being used, the organism was grown in dextrose broth, being transferred daily for three successive days. Following this, the organism was grown for a day on a phosphate agar slant, composed of 10 g. peptone, 2 g.  $K_2HPO_4$ , 1 g. dextrose, and 17 g. agar with a liter of water. A final transfer was then made to several large phosphate agar slants. By this procedure the bacteria were consistently obtained in the same state of activity.

For the phosphate agar slants, used for the final transfer, large test tubes (25-200 mm.) were employed. The culture was incubated on these for 12 hours, after which a suspension of the culture was made by washing each slant first with 2 c.c. and then 1 c.c. of a sterile salt solution, which contained KCl and  $(NH_4)_2HPO_4$  in the same concentration as the glucose media to be tested. The suspensions from all the agar slants were mixed in a sterile bottle. Tubes of media were inoculated with this in the ratio of one c.c. of suspension to 25 c.c. of media. This heavy inoculation caused the increase in acidity to begin at once, and changes in pH were evident in a few hours.

This method of inoculation seemed preferable to the use of cultures grown in peptone broth, because of the simple composition of the test media. If one c.c. of peptone broth were added in inoculating the test media, it would add nutrients that might materially affect the metabolism of the organism. Consequently, slight differences in the concentration of glucose in the test media would have less effect on the pH change in the presence of the nutrients found in peptone broth.

A well controlled method of preparing the bacterial suspension is essential, as the activity of the bacteria is one of the most important factors in determining the rate of decomposition of glucose and the subsequent increase in acidity. All conditions of incubation and preparation must be controlled, since the activity or potency of the organisms depends on many different factors, the relative importance of which are not known. Unless the bacterial suspension and method of inoculation are carefully duplicated, successive experiments will not be comparable.

Due to the fact that it was necessary to duplicate the test media, only chemically pure salts and glucose were used in preparing them. The medium used in most experiments consisted of 0.20 per cent  $(NH_4)_2HPO_4$ , 0.1 per cent KCl, glucose in varying quantities, KOH to bring the H-ion concentration to a pH of 7, and distilled water. Thus all the ions necessary for the metabolism of the organism were secured in a medium that could be reproduced. In later experiments it was demonstrated that the concentration of  $(NH_4)_2HPO_4$  could be reduced without decreasing the

rate of growth of the organism. In this way the buffer value of the medium was decreased, resulting in a greater change in pH value.

It was found convenient in most of the experiments to add bromthymol blue, as an indicator, to the media. The approximate change in pH could then be followed, enabling the more accurate electrometric determinations to be made at the time of greatest difference.

The tubes of media were sterilized in an autoclave at 15 pounds pressure for 20 minutes. The glucose in the lower concentrations used, showed no indication of caramelization. *Aerobacter levans* showed no growth in media in which sucrose was the sole source of carbon, indicating that there was no inversion of the sucrose during sterilization.

After sterilization and inoculation the tubes of media were incubated under aerobic or anaerobic conditions, depending upon conditions desired. In the aerobic incubation it was difficult to keep the media saturated with air or to maintain the same approximate concentration of air in all tubes. This was complicated by the fact that CO<sub>2</sub> was evolved, which had a tendency to prevent contact of the media with air.

In the earlier experiments 100 c.c. tincture bottles were used for incubating the media. About 50 c.c. of media were placed in each bottle, and after inoculation the bottles were partly immersed in a constant temperature water bath, held at 30°C. Since only 20 c.c. of media were necessary for a pH determination, test tubes with about 50 c.c. capacity were satisfactory. After inoculating 20 c.c. of media in the tube, it was rolled between the hands and placed in the water bath, without further agitation. This precaution was taken to insure the same approximate concentration of air in each tube. In later experiments more constant aerobic conditions were obtained by the use of 50 c.c. Erlenmeyer flasks, using 25 c.c. of media, since a greater surface area was exposed. After inoculation the flasks were shaken and placed in the thermostat.

Anaerobic conditions were obtained by slowly bubbling nitrogen through large hard glass test tubes of 50 c.c. capacity containing 35 c.c. of the media. The tubes were fitted with rubber stoppers, through which a short and a long bent glass tube were passed; by means of these tubes nitrogen could be bubbled through the media continually. Since it was impossible to flame the rubber stoppers effectively, these tubes of media were inoculated immediately after sterilization, then placed in the thermostat, and the long bent tube connected with a source of nitrogen.

The approximate change in pH of media was followed by the color change of the bromthymol blue. The final determination of pH was made by means of a quinhydrone electrode.

The rate of pH change varied with the activity of the organism, the buffer value of the medium, and the original concentration of the sugar. Because of these conditions, the optimum period of incubation for a glucose determination varies from two to 24 hours for aerobic conditions and from four to six days for anaerobic conditions.

#### EXPERIMENTAL

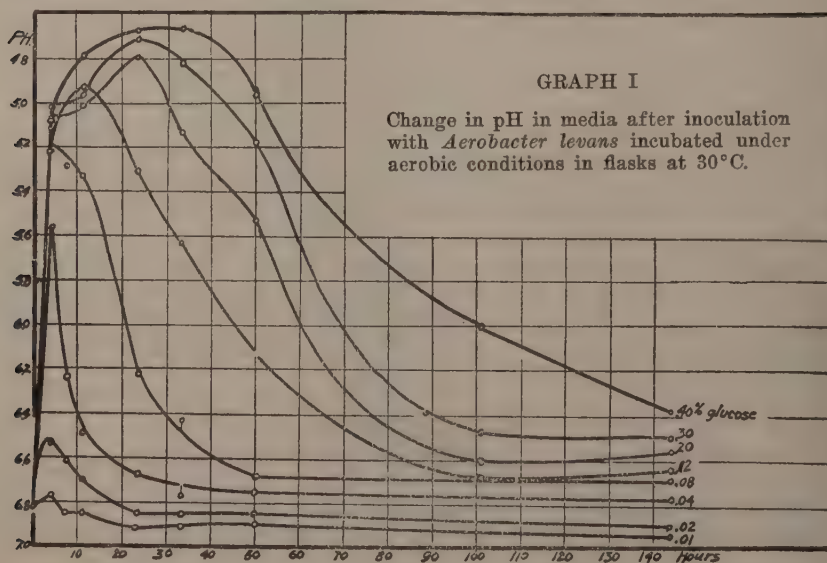
##### EFFECT OF GLUCOSE CONCENTRATION ON pH CHANGES OF MEDIA

Table 1 and Graph I give data typical of the series of pH changes that all media used pass through following inoculation with *Aerobacter levans*

and incubation under aerobic conditions. Immediately after inoculation the pH of the medium decreases, due to the decomposition of glucose into organic acids. The rate of this change is dependent on the temperature of incubation, the buffer value of the medium, and the activity of the organism. After a few hours the media containing low concentrations of glucose began to show an increase in pH, due to the decomposition of the organic acids formed by the organism. The media containing higher concentrations of glucose continue to decrease in pH, but at a slower rate, indicating that simultaneously with the change of glucose into acids, these acids are being decomposed again. The time required for the media to attain a minimum pH, as well as the rate with which the pH value increases afterwards, is dependent on the original concentration of glucose. In other words, two possibilities are suggested for determining glucose concentration with this organism, the time required for the medium to reach a minimum pH value, or the relative pH value of the media at a time after the minimum pH value has been passed.

TABLE 1. *Change in pH in Media after Inoculation with Aerobacter levans and Incubations in Flasks under Aerobic Conditions.*

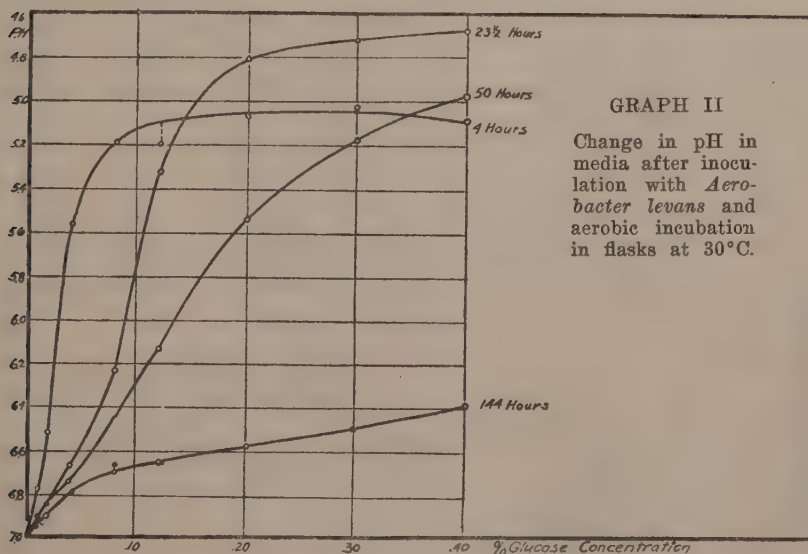
Original conc. of glucose	Original pH	pH developed after hours incubation							
		4 hrs.	7.5 hrs.	11.25 hrs.	23.5 hrs.	33.5 hrs.	50 hrs.	101 hrs.	144 hrs.
.01	6.82	6.77	6.85	6.85	6.92	6.91	6.90	.....	6.95
.02	6.82	6.53	6.61	6.70	6.85	6.85	6.85	.....	6.90
.04	6.82	5.56	6.24	6.49	6.67	6.77	6.75	.....	6.78
.08	6.82	5.19	5.29	5.32	6.22	6.44	6.68	.....	6.70
.12	6.82	5.22	.....	4.93	5.32	5.64	6.13	6.70	6.65
.20	6.82	5.08	.....	5.02	4.80	5.14	5.54	6.61	6.57
.30	6.82	5.02	.....	4.97	4.72	4.83	5.18	6.44	6.51
.40	6.82	5.09	.....	4.80	4.68	4.67	4.97	6.00	6.39





Although the type of graph shown is general for all experiments, the exact time at which the difference in pH is greatest, varies in duplicate media with the activity of the organism. Although this factor has been controlled by preparing the emulsion as described, in most work it would be more practicable to make control experiments, using the same emulsion in known and unknown solutions.

It will be noted from Graph I that there is no one time at which the pH values of the media are directly proportional to the original concentration of glucose. The media that originally contain 0.01 per cent and 0.02 per cent glucose vary appreciably in pH only from the fourth to the twelfth hour, while the media containing more than 0.12 per cent glucose are almost identical in pH value until after this time. The best period of incubation to distinguish between media containing 0.04 per cent and 0.08 per cent is between the seventh and twentieth hour. The media containing from 0.08 per cent to 0.30 per cent differ most from the twenty-fourth to the forty-eighth hour, and the 0.30 per cent and 0.40 per cent media are identical, within experimental error, until after this. These differences can be seen more clearly from a study of Graph II, in which the pH values attained after specified periods of incubation are plotted against the original concentration of sugar. The data used are the same as in table 1 and Graph I.



From a study of this graph, it is evident that at the end of four hours there is no difference in pH value of the media containing 0.08 per cent to 0.40 per cent glucose, although this is the only time when the 0.01 per cent and 0.02 per cent media vary appreciably. The media containing 0.04 per cent to 0.20 per cent vary most in pH after 23½ hours, while the 0.30 per cent and 0.40 per cent media do not vary greatly in pH until 50 hours have passed.



## EFFECT OF OXYGEN SUPPLY ON pH CHANGE

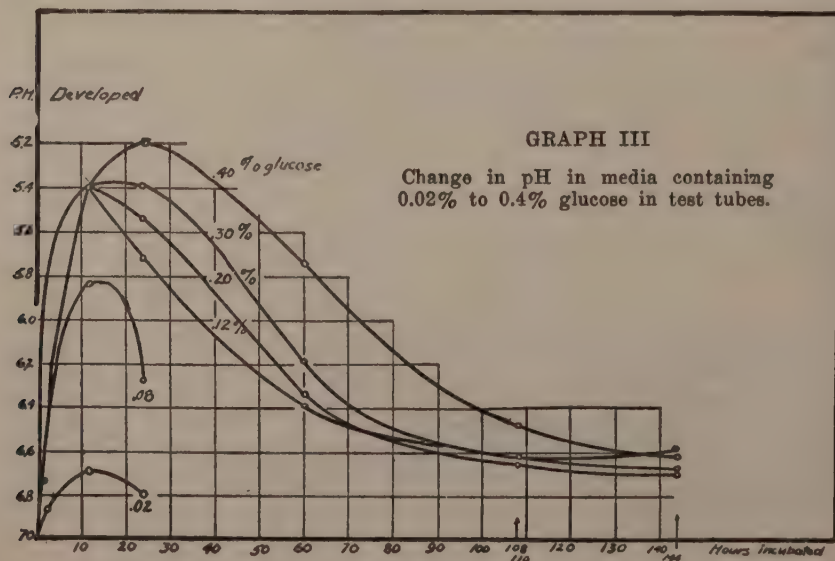
At different periods of the work, test tubes, bottles and Erlenmeyer flasks were used for incubating the media. It would be expected that the type of change in pH in each of these would differ since the amount of oxygen available to the organism would vary. Since oxygen is necessary for the decomposition of acids formed from the glucose by the organisms, these acids disappear more slowly in tubes in which only a small surface of the medium is exposed to the air. Saturation with oxygen would be difficult because carbon dioxide would be evolved by the organism, thus forming a layer of this gas over the media, which would retard the absorption of the air.

When Erlenmeyer flasks were used to incubate the media, the conditions were more nearly aerobic and the results obtained appeared more consistent. It is quite probable that the air dissolved in the media was at all times sufficient for the aerobic growth of the organism.

With the use of test tubes, there was an approximation of anaerobic conditions. The results obtained with test tubes were not as consistent as with flasks, probably because the supply of oxygen was a limiting factor. Typical results obtained from the use of test tubes are shown in table 2 and Graph III.

TABLE 2. *Change in pH in Media Containing .02 to .4 per cent Glucose when Incubated in Test Tubes.*

Original conc. of glucose	Original pH	pH developed after incubation					
		2 hrs.	12 hrs.	24 hrs.	60 hrs.	108 hrs.	6.5 days
.02	6.90	6.88	6.70	6.80			
.08	6.90	6.75	5.84	6.29			
.12	6.84		5.40	5.73	6.40	6.64	6.70
.20	6.84		5.40	5.65	6.37	6.67	6.72
.30	6.84		5.40	5.40	6.20	6.64	6.66
.40	6.84		5.40	5.20	5.75	6.48	6.63



When 100 c.c. tincture bottles were used to incubate the media, the results were intermediate between those obtained by the use of test tubes and by the use of flasks. Since the flasks seemed to offer more nearly aerobic conditions, they were used entirely in the latter experiments.

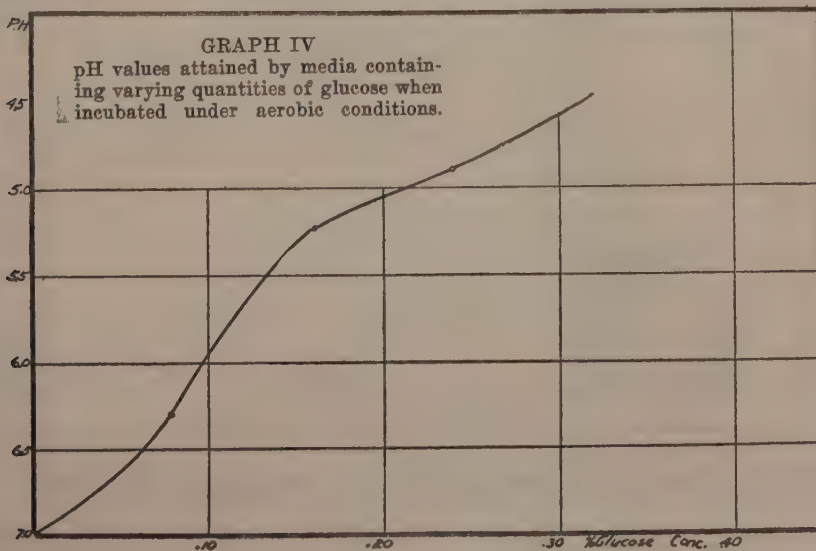
#### EFFECT OF ANAEROBIOSIS ON pH CHANGE

Since it is only under aerobic conditions that the bacteria can decompose the acids, under anaerobic conditions there will be no subsequent increase in pH. The pH values of the media, after growth has discontinued, will then be determined by the total acid produced. These values are probably not dependent on the rate of growth of the bacteria, or on any of the factors determining this, and should vary only with the amount of glucose that has been decomposed.

As one would expect, the pH values of the media decrease at a rate that is independent of the concentration of the glucose, until a constant pH is reached. From the results obtained from changes under usual aerobic conditions, one might infer that the glucose would have disappeared, and the media would have reached constant minimum pH values within 24 hours in all solutions. On the other hand, the increase in acidity and the decrease in glucose concentration would decrease the number of living bacteria and also the rate of decomposition of the remaining glucose. As a rule, the pH values of media containing less than 0.40 per cent glucose were constant after four to six days incubation. Some typical results are shown in table 3 and Graph IV.

TABLE 3. *Change in pH of Media under Anaerobic Conditions.*

Original conc. of glucose	Original pH	Final pH of media after incubation under anaerobic conditions				Average
.08	6.63	6.25	6.27	6.27	6.36	6.29
.16	6.63	5.41	5.49	5.46	5.43	5.45
.24	6.63	4.93	4.90	4.88	4.87	4.90
.32	6.63	4.55	4.62	4.43	4.58	4.52



Since only a few tubes of media could be tested at a time, and since four to six days were required for one incubation, it was not possible to run as many varied tests as under aerobic conditions. The results given are typical of several experiments run under identical conditions.

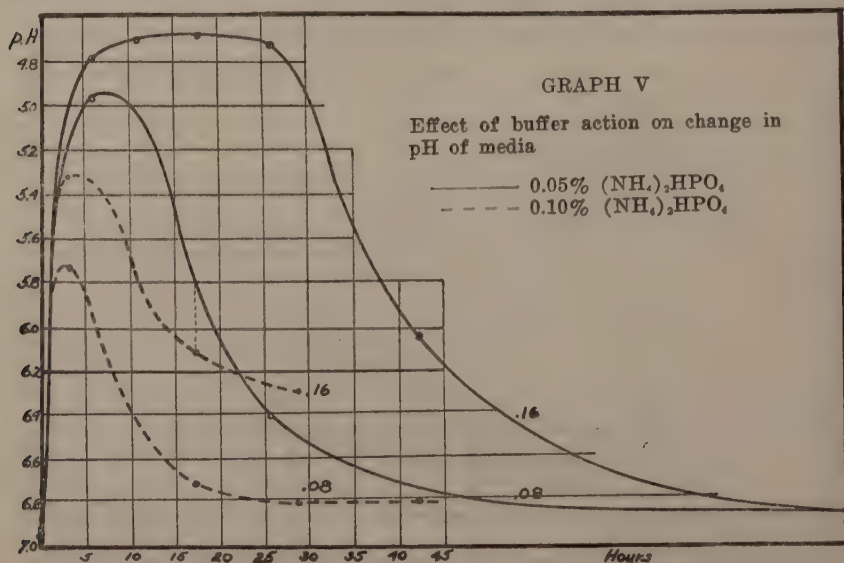
Although requiring more time, a method of determining glucose based on anaerobic conditions would probably be more dependable, since only the glucose originally present would produce a change in pH value. It would be especially valuable for the determination of glucose in media containing lactic acid, or other organic compounds that decompose under aerobic conditions, and consequently have a decided effect on the pH value.

#### EFFECT OF BUFFER VALUE OF MEDIA ON pH CHANGE

The  $(\text{NH}_4)_2\text{HPO}_4$  added to the media acts not only as a source of nitrogen for the organism, but also as a buffer, decreasing the rate of change in pH value. The concentration of this salt actually required by this organism as food is much less than the 0.20 per cent or 0.10 per cent concentration that was ordinarily used in the preparation of the media. As a result, it is possible to increase the rate of change in pH value without

TABLE 4. *Effect of Buffer Action on Change in pH of Media.*

Original conc. of glucose	pH of media containing .05% $(\text{NH}_4)_2\text{HPO}_4$						pH of media containing .10% $(\text{NH}_4)_2\text{HPO}_4$		
	8 hrs.	6 hrs.	19 hrs.	27 hrs.	46 hrs.	92 hrs.	3.5 hrs.	19 hrs.	30 hrs.
.04	5.83	6.30	6.83	6.87	7.00	6.99			
.08	5.32	4.93	6.12	6.39	6.80	6.85	5.73	6.72	6.82
.12	5.32	4.97	5.21	5.38	6.46	6.83			
.16	5.32	4.97	4.92	4.93	6.05	6.85	5.32	6.10	6.30
.24							5.32	5.31	6.03
.32							5.32	5.18	5.31



affecting the rate of acid production in the medium by using a smaller concentration of this salt.

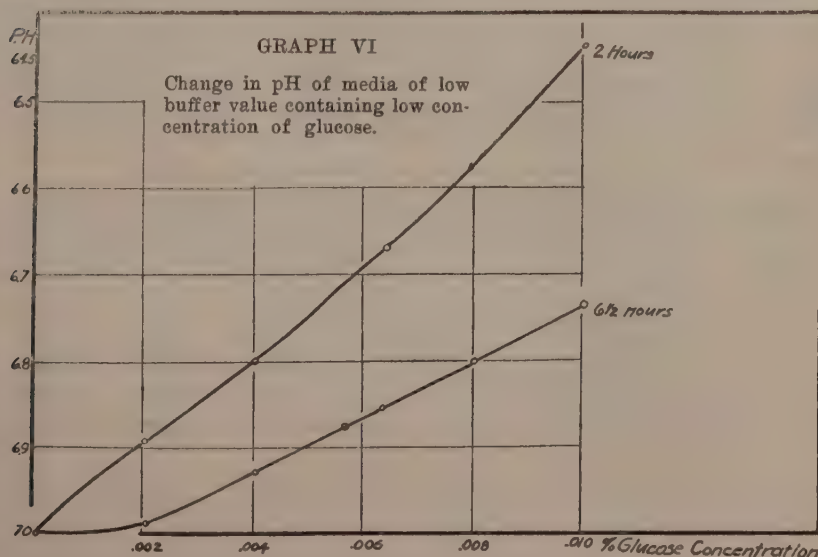
In order to determine the minimum ammonia concentration necessary for the organism, its concentration in the media after incubation was determined by the colorimetric method described by Folin and Bell (5). After three days of incubation under aerobic conditions, the loss of ammonia was scarcely detected by this method. When only 0.05 per cent  $(\text{NH}_4)_2\text{HPO}_4$  was used in the media about one-sixth of the ammonia had disappeared in two to three days. The results obtained by the use of media similar to those in former experiments, using 0.05 per cent instead of 0.10 per cent  $(\text{NH}_4)_2\text{HPO}_4$ , are shown in table 4 and Graph V.

In the less buffered media, the pH value diminishes at a more rapid rate, and the maximum change in pH is about twice as great as in the identical media containing the higher concentration of  $(\text{NH}_4)_2\text{HPO}_4$ . This indicates that in experiments on low concentrations of glucose it would be advantageous to use less buffered media, which would require less time for a pH change and make it possible to determine the glucose concentration earlier.

The possible use of lightly buffered media in distinguishing between low concentrations of glucose is demonstrated in table 5 and Graph VI.

TABLE 5. *Change in pH of Media of Low Buffer Value Containing Low Concentrations of Glucose.*

Original concentration of glucose	Original pH	pH of media after incubation	
		2 hours	6.5 hours
.002	7.00	6.88	6.99
.004	7.00	6.80	6.93
.006	7.00	6.68	6.87
.008	7.00	6.57	6.80
.010	7.00	6.44	6.73





The media used were similar to those in former experiments, except that it contained only 0.02 per cent  $(\text{NH}_4)_2\text{HPO}_4$  and 0.002 per cent to 0.01 per cent of glucose. Readings were taken in a few hours, since the pH value changed rapidly.

The pH values of the media were proportional to the glucose originally present at the end of two hours. It is possible that the low glucose concentration was a limiting factor in determining the rate of growth of the organism, and the rate of acid production. This would be in agreement with the observations of Slator (10), (11), that in low sugar concentrations the rate of carbon dioxide production is a function of the concentration of the sugar. It is possible also that the bacteria had assimilated the sugar present, and were beyond the point of their lowest pH value, after a two hour incubation period.

The results obtained with these media are more consistent than in former experiments, as when plotted they approach a straight line. This may be due to the fact that in the short time of incubation the air absorbed in all media is in excess of that required, and the action of the organisms is more consistent.

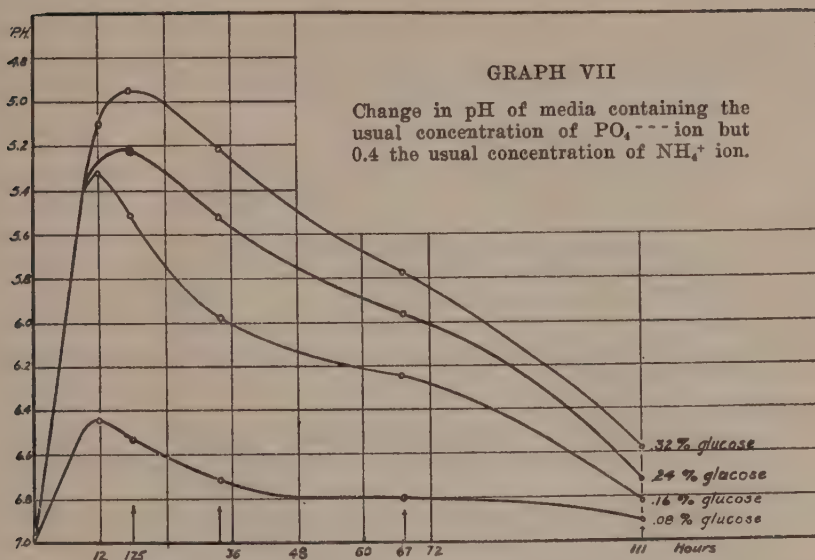
From a study of these results and of those obtained by Kendall and Yoshida (7), it would seem as if this biological method of determining sugars would be most applicable to concentrations of less than 0.01 per cent of sugar. The increase in accuracy is not enough, however, to make it advisable to dilute glucose solutions of higher concentrations. It should also be noted that determinations of these low concentrations of glucose are possible only in solutions in which the buffer value is very low. This is not the case in many biological fluids or other solutions that one might wish to analyze.

#### EFFECT OF INCREASE OF PHOSPHATE AND DECREASE OF AMMONIUM ION ON pH CHANGE

Besides diminishing the buffer value, a decrease in the concentration of  $(\text{NH}_4)_2\text{HPO}_4$  might influence the metabolism of the organism favoring the decomposition of either the acids or the sugars present. In order to determine the effect of ammonium ion concentration, a medium was prepared with other constituents in the same proportion as earlier experiments, but with only 0.04 per cent  $(\text{NH}_4)_2\text{HPO}_4$  and with 0.06 per cent  $\text{H}_3\text{PO}_4$  added to make the buffer value about the same as before. The pH was adjusted to a value of seven by adding KOH. The results obtained are shown in table 6 and Graph VII.

TABLE 6. *Change in pH of Media Containing 0.04%  $(\text{NH}_4)_2\text{HPO}_4$  and  $\text{H}_3\text{PO}_4$ .*

Original conc. of glucose	Original pH	pH of media after incubation				
		12 hrs.	17.5 hrs.	34 hrs.	67 hrs.	111 hrs.
.08	7.02	6.44	6.54	6.72	6.80	6.92
.16	7.02	5.32	5.52	5.98	6.25	6.83
.24	7.02	5.11	5.22	5.52	5.97	6.73
.32	7.02	5.12	4.95	5.22	5.78	6.58



Graph VII is similar to those in the preceding experiments. The increase in pH may be slightly slower than in media containing more ammonia, but the difference is not marked.

#### SUMMARY AND CONCLUSIONS

1. The rate of lowering of the pH of media, containing over 0.01 per cent glucose when inoculated with *Aerobacter levans*, is not proportional to the glucose concentration.

2. The secondary change, under aerobic conditions, is an increase in pH due to the destruction of the acids formed from the sugar, and is a function of the glucose concentration.

3. Under anaerobic conditions, there is no destruction of acids, and the total pH change is a function of the glucose concentration.

4. When the glucose concentration is less than 0.01 per cent, and the buffer value is low, the initial lowering of pH is a function of the glucose concentration.

5. Media varying from 0.002 per cent to 0.40 per cent in glucose concentration have been studied by varying the buffer value.

6. The results demonstrate a possibility of determining glucose and other sugars in small concentration by a biological method.

## LITERATURE CITED

1. BOURQUELOT, EM.  
1901. Recherches, dans les vegetaux, du sucre de canne a l'aide de l'invertine et des glucosides a l'aide de l'emulsine. *Compt. Rend. Acad. Sci. Paris.* 133:690-692.
2. BOURQUELOT, EM. ET H. HERISSEY.  
1912. Du choix de la levure dans l'application des procedes biochimiques a la recherche des sucres et des glucosides. *Jour. Pharm. chim.; ser. VII,* 6:246-253.
3. CASTELLANI, R. AND F. E. TAYLOR.  
1917. Mycological detection and determination of certain carbohydrates and other carbon compounds in pathological work. *Brit. Med. Jour.* 1917; 2:855-857.
4. DAVIS, W. A.  
1916. The use of enzymes and special yeasts in carbohydrate analysis. *J. Soc. Chem. Ind.* 35:201-210.
5. FOLIN, O., AND R. D. BELL.  
1917. Applications of a new reagent for the separation of ammonia in urine. *Jour. Biol. Chem.* 29:329-335.
6. KENDALL, A. I.  
1923. Carbohydrate identification by bacterial procedures. *Jour. Infect. Dis.* 32:362-368.
7. KENDALL, A. I. AND S. YOSHIDA.  
1923. The estimation of small amounts of carbohydrates by bacterial procedures. *Jour. Infect. Dis.* 32:355-361.
8. PASTEUR, L.  
1858. Memoire sur la fermentation de l'acide tartrique. *Compt. Rend. Acad. Sci. Paris.* 46:615-619.
9. POTTEVIN, H.  
1903. Influence de la configuration stereochimique des glucosides sur l'activite des diastases hydrolytiques. *Ann. Inst. Pasteur.* 17:31-51.
10. SLATOR, A.  
1906. Studies in fermentation I. The chemical dynamics of alcoholic fermentation by yeast. *Jour. Chem. Soc.* 89:128-142.
11. \_\_\_\_\_.  
1908. Studies in fermentation II. Mechanics of alcoholic fermentation. *Jour. Chem. Soc.* 93:217-242.

# THE NORMAL VARIABILITY OF THE SEX-RATIO IN THE GUINEA PIG

R. G. SCHOTT AND W. V. LAMBERT

*From the Department of Genetics, Iowa State College\**

Accepted for publication January 20, 1930

Sex in mammals has been shown to be determined primarily by the heterogametic potentialities of the male. Parkes (1924) has summarized the genetic and cytological evidence on this question as well as the data on the sex-ratio in mammals. He lists the guinea pig among heterogametic mammals, but there is no genetic evidence to support this; neither has bimodality been observed in size distributions of sperm. Painter (1926) has advanced cytological evidence which places the guinea pig among those mammals in which heterogamy is still in question.

Various environmental factors are thought to have differential effects on the sexes, causing variations in the sex-ratio at any given time. One of the more common environmental agents thought to affect sex determination is the physiological condition of the parents, either one or both. If the sex is determined at fertilization, such influences would have to operate previous to fertilization, and operate differentially on the X and Y bearing germ cells. After fertilization any change in the ratio would depend on the relative viability of the two sexes.

The material considered here is taken from laboratory records on a guinea pig colony that has been maintained by the Department of Genetics at Iowa State College. Observations were made to include the young born from January, 1922, when the colony was started, until January, 1929.

The data are tabulated so that consideration of the sex-ratio as affected by seasons, age of parents, litter frequency and size, and viability of the sexes can be made directly. The sex-ratio used herein expresses percentage of males.

The hypothesis that seasonal changes might have an effect on the sex-ratio has been one of long standing. In rodents like the guinea pig, which are quite sensitive to seasonal fluctuations in spite of fairly uniform laboratory conditions, one would expect a noticeable expression of such effects. In table 1 the ratios are given by consecutive months, the total for each month being the sum of the observations for that month, during the seven years in which these data have been collected. These results give directly the ratio in any season.

As may be seen from the totals in table 1, the ratio of males to females for the whole population over the entire period is approximately equal, the proportion of males to females being 49.45. In no month is the deviation from equality large enough to be considered statistically significant when the difference is compared to its probable error.

---

\*Paper No. 33 from the Department of Genetics.



TABLE 1. *Sex-ratio Listed by Months. Observations Were Made from January, 1922, to January, 1929.*

Month	♂♂	♀♀	Totals	Per cent ♂♂	Dev. P.E.
Jan.	65	49	114	57.02±3.15	2.22
Feb.	64	58	122	52.46±3.05	0.80
March	65	81	146	44.52±2.78	1.97
April	41	48	89	46.07±3.57	1.10
May	72	62	134	53.73±2.91	1.28
June	80	80	160	50.00±2.66	0.00
July	88	95	183	48.09±2.49	0.76
Aug.	114	118	232	49.14±2.21	0.38
Sept.	80	94	174	45.98±2.55	1.57
Oct.	129	104	233	55.39±2.20	2.43
Nov.	112	144	256	43.75±2.10	2.97
Dec.	86	85	171	50.29±2.57	0.11
Totals	996	1018	2014	49.45±0.23	2.39

When considering the fluctuations of the ratio from month to month, the differences occurring from April to September are less extreme than those occurring from October to March, as shown by figure 1. If one tests the groups appearing in any two months for independence, using the  $X^2$  method given by R. A. Fisher (1928, p. 82-84), the extreme cases of January against November show a value for P of .01, which could be taken as significant of a difference between the two groups. However, when January, with its ratio of 57.02, is tested against December, which shows a ratio of 50.29, the  $X^2$  value of 1.24 with a P of .18 does not indicate a significant difference between the two groups. Since December represents the group directly between January and November, and since this classification is entirely arbitrary, the apparent extreme fluctuations must mean that variation approaching significance can occur in short periods of time.

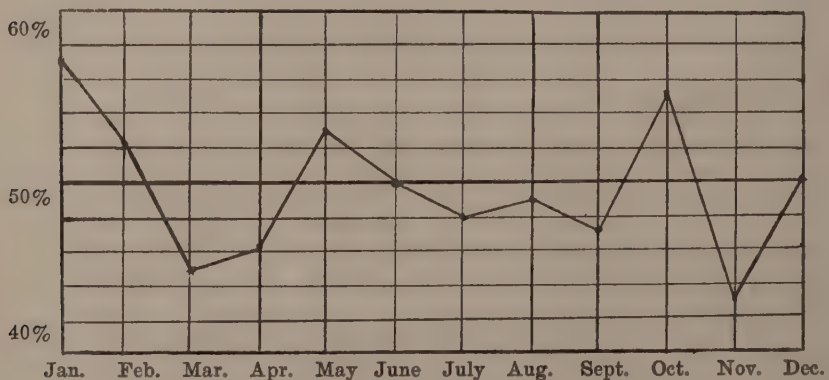


FIG. 1. Showing the fluctuation of the sex by month intervals.

If this population is grouped into summer and winter divisions, taking as summer the months from April to September, during which period

least monthly fluctuations occur, and the period from October to March as the winter season, the ratio for the summer group is 48.8, while that of the winter group is 50.0. Upon testing these two groups for independence by the above method, the  $X^2 = .223$  and  $P = .69$ . This result shows conclusively that the two groups do not represent independent classes.

When the data are arranged in order of consecutive years as in table 2, little variation appears from one year to the next, and no extreme deviations from the expected ratio of equality occur. The apparent extreme ratio in 1922 is due to the small numbers involved.

TABLE 2. *Sex-Ratio Tabulated by Consecutive Years*

Year	♂♂	♀♀	Totals	Per cent ♂♂	Dev. P.E.
1922	24	43	67	35.82±4.11	3.45
1923	101	98	199	50.75±2.38	0.31
1924	137	127	264	51.89±2.07	0.91
1925	132	123	255	51.67±2.10	0.83
1926	310	325	635	48.83±1.33	0.87
1927	182	173	355	51.27±1.78	0.71
1928	110	129	239	46.03±2.17	1.82

TABLE 3. *Sex-Ratio Appearing According to Consecutive Age Groups of Sire. Beginning at 100 Days Each Age Group Includes 110 Days. Age is taken at the time the Litter Was Sired.*

Sires' age in days	♂♂	♀♀	Totals	Per cent ♂♂	Dev. P.E.
100	50	47	97	51.54±3.42	0.45
210	186	206	392	47.44±1.70	1.50
320	204	207	411	49.63±1.66	0.22
430	219	196	415	52.77±1.65	1.67
540	145	146	291	49.82±1.97	0.09
650	79	102	181	43.64±2.50	2.14
760	48	54	102	47.05±3.33	0.88
870	34	28	62	54.83±4.28	1.12
980	31	32	63	49.20±4.24	0.18

If the male in the guinea pig produces two types of spermatozoa, it is conceivable that changes in the physiological condition of the male might sometimes differentially influence the viability of the X or Y bearing sperm cell. If the males' reproductive physiology changes, or if the general physiological changes with aging have a differential effect on the two kinds of germ cells, it should be expressed in the offspring of sires of different ages or of the same sire at different ages. Table 3 shows the ratio of the sexes appearing for consecutive age groupings of the sires. The sex-ratio is so remarkably constant for the various age groups that no age influence upon the sex-ratio can be postulated from these data.

As with the sires, the age of the dam has no apparent influence on the sex-ratio. These data are shown in table 4. Here, however, one would expect, in addition to physiologic changes accompanying age, the effects of pregnancies.

TABLE 4. *Sex-Ratio by Consecutive Age Groups of Dams. Age is Taken at Time of Farrowing.*

Dam's age in days	♂♂	♀♀	Totals	Per cent ♂♂	Dev. P.E.
100	16	19	35	45.71±5.69	0.45
210	247	229	476	51.89±1.54	1.50
320	234	238	472	49.58±1.54	0.22
430	196	190	386	50.78±1.71	0.06
540	137	148	285	48.07±1.99	0.09
650	61	66	127	48.03±2.99	0.06
760	49	57	106	46.23±3.27	0.88
870	24	25	49	48.98±4.81	0.21
980	32	46	78	41.02±3.81	2.35

TABLE 5. *Sex-Ratio by Sequence of Litter.*

Litter sequence	♂♂	♀♀	Totals	Per cent ♂♂	Dev. P.E.
1	272	269	541	50.27±1.44	0.23
2	232	230	462	50.22±1.56	0.14
3	161	154	315	51.11±1.89	0.58
4	119	144	263	45.25±2.07	2.29
5	89	78	167	53.29±2.60	1.26
6	44	55	99	44.44±3.38	1.64
7	37	37	74	50.00±3.91	0.00
8	19	14	33	57.58±5.86	1.29
9	23	37	60	38.33±4.35	2.68

The sequence in which litters are produced should roughly correspond to the age of the dams. As table 5 shows, the total number of young appearing after the fourth pregnancy is small. The dams that produce over four litters are advanced in age. If a differential effect of the physiological processes of the body of the mother is reflected on the sex of the young in utero, it should be evident from the data in tables 4 and 5. Unfavorable physiological conditions may be reflected on the young by foetal resorption, and if male embryos among the mammals are more susceptible to adverse conditions, as certain evidence from observations on man and the rat seems to indicate (Parkes, 1924), then the sex-ratio should be disturbed. However, since the birth ratio is not markedly irregular in these data, one would have to assume that there is a greater percentage of males in the primary or conception ratio to allow for the secondary ratio or birth equality. Ibsen (1928) has shown that foetal resorption is of frequent occurrence in guinea pigs. However, no differential action against the sexes has been shown. Ibsen (1923), and Ibsen and Summers (1928) point out that wide deviations in the sex-ratio exist for dams of different ages, but these age differences again disappeared when larger number were observed. Their general conclusion is, that in the guinea pig, the age of the dam has no significant effect on the sex ratio.

Another factor that might influence the physiological environment of the litter in utero is the interval between pregnancies. As listed in table 6, the first class, which has an interval between litters of 65 to 75 days, is composed of litters that were sired immediately after the preceding litter was

farrowed. The nursing period in the guinea pig is about 30 days and the gestation period is normally 67 days. Thus the dam was carrying the litter while nursing, and this double drain on her system might possibly affect the viability of the weaker sex. Since the ratio at birth in this group is 50:50, it seems apparent that two or more successive pregnancies does not cause a greater death rate among the male embryos.

With the next group, where the interval is 75 to 95 days, the mating must have taken place during the latter part of the nursing period. The ratio of males from these matings is low,  $44.07 \pm 2.3$  per cent, but the figure is not statistically significant, if the criterion for significance demands that the deviation from the expected (namely 50:50) divided by the P.E. must equal at least three.

In the third class, where conception occurred after the nursing period, the ratio again returns to 50:50. From these data it becomes apparent that the interval between pregnancies does not influence the normal secondary sex ratio in the guinea pig.

TABLE 6. *Sex-Ratio According to the Interval Between Successive Litters.*

Intervals between litters in days	♂♂	♀♀	Totals	Per cent ♂♂	Dev. P.E.
65-75	343	343	686	$50.00 \pm 1.2$	0.00
75-95	93	118	211	$44.07 \pm 2.3$	2.56
95-up	288	288	576	$50.00 \pm 1.4$	0.00

TABLE 7. *Sex-Ratio by Size of Litter.*

No. in litters	No. of litters	♂♂	♀♀	Totals	Per cent ♂♂	Dev. P.E.
1	204	90	114	204	$44.11 \pm 2.36$	2.49
2	398	403	393	796	$50.62 \pm 1.19$	0.52
3	213	313	326	639	$48.98 \pm 1.33$	0.76
4	69	140	136	276	$50.72 \pm 2.02$	0.35
5	15	37	38	75	$49.33 \pm 3.49$	0.19
6	4	13	11	24	$54.16 \pm 5.15$	0.80

The sex ratio for litters of various sizes is given in table 7. The only way that size of litter could affect the sex-ratio at birth perhaps would be, through differential action against the male foetuses, in those cases in which the mother was carrying a large litter. Such discrimination is not apparent from these data. The lowest ratio of males appears in litters of one. The sex ratio for litters of two, three, four, and five is close to equality. For litters of six the total number is too small to furnish conclusive evidence, although the excess is in favor of the males.

A comparison of the relative viability of the two sexes is given in table 8. More males are dead at birth than females, the percentage being  $55.34 \pm 2.67$ . Postnatal mortality from the first day to the fifteenth strikes the males slightly harder than the females, while during the next fifteen days the female mortality is highest. In animals living over thirty days, the percentage of males is  $48.86 \pm 0.84$ , a figure close to equality. Although



the tabulation would seem to indicate a tendency toward male weakness in the late embryonic stages, the differences in the mortality of the two sexes are not great enough to be certainly indicative of a differential sexual mortality. Ibsen (1928) found no differential mortality between the sexes.

TABLE 8. *Relative Number of Deaths Occurring in the Sexes at Birth, from the First to the Fifteenth Day, from the Fifteenth to the Thirtieth Day, and the Sex-Ratio in Animals Living Over Thirty Days.*

	♂♂	♀♀	Totals	Per cent ♂♂	Dev. P.E.
Dead at birth	88	71	159	55.34±2.67	2.00
Dead first 15 days	85	77	162	52.46±2.64	0.93
Dead 15-30 days	53	61	114	46.49±3.15	1.11
Living over 30 days	770	809	1579	48.86±0.84	1.47

As seen in table 1, the sex-ratio on the total number of animals is approximately equal. When the grouping is made according to months, considerable fluctuation is apparent. However, any grouping into larger seasonal classes tends to shift the ratio toward equality, for the fluctuations are not progressive or consistent in one direction. Ibsen (1928) reports that classification by months, with some 7,989 individuals, shows no significant variation from month to month.

In regard to sex ratios on large numbers of animals, regardless of possible modifying influences, Wright (1922) gives the sex-ratio for the guinea pigs as 101.5 males to 100 females. This ratio was based upon a total of 10,875 individuals. Ibsen and Summers (1928) found the sex-ratio on a population of 18,888 guinea pigs to be 51.4 per cent males. In both these cases the ratio is higher than that observed in this report, namely 49.45. The total sex-ratio for the three observations combined is 50.9.

#### SUMMARY

1. The sex-ratio observed in a colony of 2,014 guinea pigs was  $49.45 \pm 0.23$  per cent males.

2. The observed sex-ratio is slightly lower in summer than in winter, but it does not vary consistently from month to month.

3. Age of sire, age of dam, size of litter, interval between litters, and sequence of litters had no effect upon the sex-ratio.

4. A differential mortality rate between the sexes, from birth to the fifteenth day, and from fifteen days to thirty days, is not supported by these data.

5. No ordinary environmental factor appears to have even a slight specific effect on the sex ratio in the guinea pig.

## LITERATURE CITED

FISHER, R. A.

1928. Statistical methods for research workers. Oliver and Boyd, London. 82-84.

IBSEN, H. L.

1923. Some genetic experiments with guinea pigs and rats. Proc. of the Amer. Soc. of Animal Prod. 99-101.

---

1928. Prenatal growth in guinea pigs, with special reference to environmental factors affecting weight at birth. Jour. Exp. Zool. 51:51-91.

---

AND O. B. SUMMERS.

1928. Additional data on the sex-ratio in guinea pigs. Anat. Rec. 37:176-177.

PAINTER, T. S.

1926. The chromosomes of rodents. Science 64:356.

PARKES, A. S.

1924. The mammalian sex ratio. Biol. Proc. of the Cambridge Phil. Soc. 2:1-51.

WRIGHT, S.

1922. The effects of inbreeding and crossbreeding on guinea pigs. U. S. Dept. of Agric. Bull. 1121:12.



# THE EFFECT OF AN EXCESS OF MAGNESIUM ON THE VOLUME OF GAS LIBERATED INCIDENTAL TO THE PREPARATION OF SOME ALKYL MAGNESIUM HALIDES

HENRY GILMAN AND ROBERT E. FOTHERGILL

*From the Chemical Laboratory of Iowa State College*

Accepted for publication February 1, 1930

## INTRODUCTION

In the preparation of alkylmagnesium halides a number of side reactions occur in addition to the following reaction:



Chief among the secondary reactions is that leading to the formation of transient free radicals which can then couple or disproportionate as follows<sup>1</sup>:



Generally, the extent of such side-reactions is limited so that most organomagnesium halides can be conveniently prepared in yields of 90 per cent and upwards. For example, it is possible to prepare *n*-butylmagnesium bromide in a 94 per cent yield<sup>2</sup>. However, some unpublished studies show that *n*-butylmagnesium bromide can be prepared in quantitative yields when three atom equivalents of fine magnesium instead of the customary one atom equivalent is used. These excellent yields, which might have been predicted in part and which are in complete harmony with the need of an excess of magnesium for the preparation of some  $RMgX$  compounds<sup>3</sup>, naturally merit confirmation by some method other than the acid-titration of Gilman, Wilkinson, Fishel and Meyers<sup>4</sup>.

Such verification was the chief purpose of the present study, and we elected the related method of Gilman and Fothergill<sup>1</sup> as a check on the correctness of the new extremely high yields. Obviously, however, their method involving the measurement of evolved gas could not be applied to *n*-butylmagnesium bromide because with this compound the coupling product (*n*-octane, see Reaction (III) ) is not gaseous under ordinary experi-

<sup>1</sup>Gilman and Fothergill, *J. Am. Chem. Soc.*, **50**, 3334 (1928). This article contains references to other pertinent studies, particularly those of Gomberg and co-workers.

<sup>2</sup>Gilman, Zoellmer and Diekey, *ibid.*, **51**, 1576 (1929). See, also, Gilman and McCracken, *ibid.*, **45**, 2462 (1923).

<sup>3</sup>We refer to such compounds as allylmagnesium bromide, *tert*-butylmagnesium chloride, and, recently, benzohydrylmagnesium chloride.

<sup>4</sup>Gilman, Wilkinson, Fishel and Meyers, *J. Am. Chem. Soc.*, **45**, 150 (1923).



mental conditions. Accordingly, a study was made of the gases evolved incidental to the preparation of methylmagnesium iodide and ethylmagnesium bromide. Each of these two reagents was prepared with one and with three equivalents of magnesium, respectively.

If the yield of all alkylmagnesium halides is increased with the use of an excess of magnesium, then there should be less gas evolved when an excess of magnesium is used. This was confirmed in the case of ethylmagnesium bromide. However, it found no support in the experiments with methylmagnesium iodide. We have, at this time, no satisfactory explanation for the unexpected results with methylmagnesium iodide. Possibly, the yields of this organomagnesium halide are already so high that the effect of an excess of magnesium is not appreciable. This, however, cannot be a sole reason because actually more gas was evolved with three equivalents of magnesium than with one equivalent of magnesium. It is quite likely that the so-called abnormal results with methylmagnesium iodide may be due to the iodine used as a catalyst and to the iodine which is slowly and spontaneously split from this, as well as many other iodides. This iodine has an erratic action on the yields of  $\text{RMgX}$  compounds. This is strikingly emphasized in some unpublished studies concerned with the effect of varying quantities of iodine on the yields of organomagnesium halides<sup>5</sup>. A definite answer to this problem will probably be found in a study of the effect of varying quantities of magnesium on the gas evolved in the preparation of *n*-propylmagnesium bromide<sup>6</sup>.

#### EXPERIMENTAL PART

The methyl iodide and ethyl bromide were dried over anhydrous calcium chloride and redistilled. The middle fractions were used in this study. The magnesium turnings and powder were the ordinary high grades used in earlier related studies. Prior to each experiment, the apparatus was carefully dried by passing a current of dry air through it for 30 to 45 minutes.

The alkyl halide (0.1 mole) dissolved in 80 c.c. of anhydrous ether was added slowly (1.0 to 1.75 hours) to the magnesium, to which 0.1 g. of iodine had been added. When all the alkyl halide had been added, the solution was refluxed for one hour to expel the gas dissolved in the solution. Using the technique of the earlier study by Gilman and Fothergill<sup>1</sup> (with the exception that the gas was not analyzed), the gas was collected in a large eudiometer containing fresh water as the confining liquid, and allowed to stand for three hours with occasional shaking, in order to remove ether vapor. The gas was then measured in a gas burette after all the ether

---

<sup>5</sup>These studies were first started with *tert*-butylmagnesium chloride in connection with the capture of free radicals. With this organomagnesium chloride, the yields are maximum with an extremely small quantity of iodine and with one full equivalent of iodine, in the presence of an excess of magnesium to form the hypothetical magnesium iodide. Variations in yield have also been observed with varying quantities of iodine in the preparation of *n*-butylmagnesium bromide. It is also interesting to note that the latter reagent, as well as phenylmagnesium bromide, can now be prepared in quantitative yields with but one equivalent of magnesium and an optimal quantity of iodine.

<sup>6</sup>The quantities of gas evolved in differently sized runs will be measured, because it appears that the gas evolved varies somewhat with the size of a run.

vapor had been removed by washing with fresh water in an absorption pipette. The volumes given in Table 1 are those after conversion to standard conditions. The 30-80 mesh magnesium powder was that which gave a quantitative yield of *n*-butylmagnesium bromide by acid-titration.

TABLE 1.

Exp. No.	Alkyl halide	Mole	Magnesium		Time of addition in hours	Gas evolved in c.c.	c.c. differ- ence be- tween differ- ent quanti- ties of mag- nesium
			Form	Atom			
1	Ethyl Bromide	0.1	Turnings	0.1	1.25	149.3	
2	Ethyl Bromide	0.1	80-200 Mesh	0.3	1.75	131.7	-17.6
3	Ethyl Bromide	0.1	30-80 Mesh	0.3	1.3	149.8	+0.5
4	Methyl Iodide	0.1	Turnings	0.1	1.0	156.0	
5	Methyl Iodide	0.1	80-200 Mesh	0.3	1.5	172.0	+16.0

## SUMMARY

A study has been made of the effects of varying quantities of magnesium on the volume of gas evolved incidental to the preparation of methylmagnesium iodide and ethylmagnesium bromide.



# THE PREPARATION OF FURLACRYLIC ACID<sup>1</sup>

HENRY GILMAN, R. E. BROWN, G. F. WRIGHT AND A. P. HEWLETT

*From the Chemical Laboratory of Iowa State College*

Accepted for publication February 1, 1930

## INTRODUCTION

Furylacrylic acid, its salts and other derivatives are in demand. One of the most significant commercial applications of the compound at present is as a food preservative in the Orient for "Shoyu". Any improvements in the preparation of furylacrylic acid, particularly from the point of view of lowered cost, are highly desirable. Previously, Gilman, Brown and Jones<sup>2</sup> described the preparation of the ethyl ester by means of the Claisen condensation. As a result of that study the yield of ethyl furylacrylate was increased from 36.1 per cent to 63.3 per cent. The ester is very readily hydrolyzed to the acid or its salt<sup>3</sup>.

The present study is concerned with improvements in the preparation of furylacrylic acid by means of the Perkin condensation, which starts with furfural. The acid was first prepared by Baeyer<sup>4</sup>. Later, Marckwald<sup>5</sup> modified Baeyer's directions by reducing the quantity of acetic anhydride and extending the time of refluxing from eight to eleven hours. Then Gibson and Kahnweiler<sup>6</sup> prepared the acid, using a lesser proportion of both acetic anhydride and sodium acetate. None of these authors states explicitly the yield of pure acid. In some of our experiments we have obtained yields as high as 84.3 per cent. This is a higher yield than that reported hitherto. The acid so obtained melts at 138° and one crystallization from a water-alcohol mixture is sufficient to raise the melting point to 141°.

The particular advantages of our method are not restricted to an improved yield. First, we have found that it is not necessary to acidify the reaction mixture with the customarily used hydrochloric acid, inasmuch as the furylacrylic acid (under our conditions) is free directly on hydrolysis of the reaction product by water. This, naturally, effects a saving in cost because an inorganic acid is not required to liberate the furylacrylic acid. Furthermore, the product obtained when hydrochloric acid is not

---

<sup>1</sup>This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge help from the Industrial Science Research Fund for the defrayal of expenses incurred in this investigation.

<sup>2</sup>Gilman, Brown and Jones, *IOWA STATE COLLEGE JOURNAL OF SCIENCE* 2, 317 (1928).

<sup>3</sup>Gilman and Wright, *ibid.*, 3, 112 (1929). This article describes the syntheses of some perfumes and flavoring extracts from furylacrylic acid.

<sup>4</sup>Baeyer, *Ber.*, 10, 355 (1877).

<sup>5</sup>Marckwald, *Ber.*, 20, 2811 (1887).

<sup>6</sup>Gibson and Kahnweiler, *Am. Chem. J.* 12, 314 (1890).



used is of a higher degree of purity, and its working-up requires fewer operations<sup>7</sup>.

Second, we have found that it is possible to use the technical furfural instead of the highly purified, freshly distilled aldehyde. With the technical or commercial furfural the yield of acid appears to be equal to that obtained when freshly distilled aldehyde is used. Also, the product so obtained is somewhat purer than that obtained with pure aldehyde<sup>8</sup>.

One other important observation was noted in the present study. It was found that the yield of acid is significantly improved with repeated fusions of the sodium acetate. This may not be due entirely to an especially anhydrous sodium acetate obtained in this way. We are inclined to believe that a part, at least, of the improvement in yield is due to the formation of small but significant quantities of sodium carbonate resulting from the repeated fusions of the sodium acetate. Any sodium carbonate so formed would probably have a positive catalytic effect like other basic compounds. It is probably for this reason that the pyridine used in some experiments had no essential effect on the yield, despite the fact that pyridine has been used as a catalyst in the preparation of the related cinnamic acid<sup>9</sup>.

Inasmuch as recrystallization of furylacrylic acid is difficult and wasteful, our object has been to obtain it directly from a reaction mixture in a form which is sufficiently pure for ordinary reactions. It is primarily for this reason that our procedure is not patterned after that of Gibson and Kahnweiler<sup>6</sup>. When we used their principle of reducing the ratio of acetic anhydride to furfural we found the acid to be inferior to that obtained by our procedure, which is described in the Experimental Part. This inferiority in quality was particularly noticeable in substitution reactions. For example, when brominated in carbon disulfide solution it gave a yield of less than one-half that obtained with the acid as prepared in accordance with our procedure. In a typical run, patterned after Gibson and Kahnweiler's<sup>6</sup> procedure we used 384 g. (4.0 moles) of furfural, 612 g. (6.0 moles) of acetic anhydride and 800 g. (10.0 moles) of sodium acetate, and obtained a 76.6 per cent yield of furylacrylic acid melting at 130°. The acid so obtained was dark in color. It will be noticed that our time of

---

<sup>7</sup>It is probable that the free acid is obtained by virtue of the acetic acid liberated from the excess of acetic anhydride on water hydrolysis. An excess of acetic anhydride appears necessary, and has been used by all who have prepared furylacrylic acid by the Perkin reaction. If it were found possible to reduce significantly the quantity of acetic anhydride (and this may come from future studies) then, of course, it would be desirable to use the less expensive inorganic acids for hydrolysis.

<sup>8</sup>A possible explanation for the high yields of furylacrylic acid when the technical aldehyde is used may be found in the excess of acetic anhydride acting as a dehydrating agent on the small quantities of water contained in the commercial furfural. Furthermore, it appears that the technical aldehyde contains some lime, and basic compounds are very effective catalysts in the Perkin condensation. The catalytic effect of the lime is obviously lost when the crude furfural is purified by distillation.

<sup>9</sup>Bacharach and Brogan, *J. Am. Chem. Soc.*, **50**, 3333 (1928). See, also, Kalnin, *Helvetica Chim. Acta*, **11**, 977 (1928). We should make clear that in protracted refluxings like those involved in the Perkin condensation the yields vary somewhat under essentially similar conditions. For this reason, it remains to be determined unequivocally whether or not pyridine, under our experimental conditions, has a distinct catalytic effect.

heating was three to five hours less than that used by Marekwald<sup>5</sup>, and our temperatures just slightly less than that used by him.

#### EXPERIMENTAL PART

The technical furfural, 384 g. (4.0 moles), was mixed with 768 g. (7.5 moles) of acetic anhydride and 768 g. (9.4 moles) of sodium acetate which was fused three times<sup>10</sup>. The mixture contained in a three-liter three-necked flask was refluxed at 146-147° (internal temperature) for six to eight hours. During the heating, stirring was continuously applied by means of an offset blade stirrer, which barely scraped the sides of the flask<sup>11</sup>. The reaction mixture was then poured into about one liter of water, and steam distilled until four liters of steam distillate were obtained<sup>12</sup>.

The residual liquor from the steam distillation was boiled for an additional ten minutes with 20 g. of Norite, and then filtered hot<sup>13</sup>. To this filtrate, of a volume approximately four liters, was then added about one kilo of cracked ice in order to facilitate crystallization. The precipitated or crystallized furylacrylic acid was then washed with two 30 c.c. portions of cold water and dried about two feet over a steam plate<sup>14</sup>. The yield of acid obtained in this way is 400-465 g. or 73-84 per cent of the theoretical amount. It is conveniently recrystallized from a water-alcohol solution, and when so purified melts at 141°. In this crystallization, the acid is first suspended in water heated at 70°-80°; then 95 per cent alcohol is added until solution is almost complete; and, when complete solution is subsequently effected by heating the water-alcohol mixture to a boil, the solution is filtered rapidly through cheese-cloth.

The data given in Table 1 illustrate the effect on the yield of repeated fusions of the sodium acetate. The yields of the first 9 experiments are based on a furylacrylic acid melting at 138°. The yields in Experiments 10 and 11 as shown in Table 1 are based on a purified acid melting at 141°.

---

<sup>10</sup>Table 1 at the end of the Experimental Part shows the effect on the yield of one, two and three fusions of the sodium acetate.

<sup>11</sup>Some preliminary, orienting experiments showed that stirring improved the yield.

<sup>12</sup>This distillate contains acetic acid and any unchanged furfural.

<sup>13</sup>The filtration is best effected by means of a cheese-cloth filter. This permits of rapid filtration, which is highly desirable because of the marked insolubility of furylacrylic acid in warm water. Slower filtration permits cooling with consequent crystallization and clogging of ordinary filter paper.

<sup>14</sup>Rapid drying results in a slight darkening of the acid, and the compound so obtained melts about 10° lower than the product which is obtained by slow and moderate heating. In this connection it is interesting to note that when hydrochloric acid is used in hydrolysis the furylacrylic acid is somewhat darkened in color and melts lower than the product obtained when no hydrochloric acid is used in the hydrolysis.

TABLE 1. *Effect of Repeated Fusions of Sodium Acetate on the Yield of Furylacrylic Acid.*

Experi- ment No.	Number of fusions of so- dium acetate	c.c. of pyridine added as catalyst	Precipitant	Hours of refluxing	Yield in percent
1	1	None	None	8	66.6
2	1	"	"	8	70.5
3	1	"	HCl	8	79.6
4	2	"	None	8	77.0
5	2	"	"	8	69.6
6	2	"	HCl	8	76.6
7	3	"	HCl	6	82.6
8	3	"	None	8	73.5
9	3	12 c.c.	"	8	84.3
10	3	None	HCl	8	70.0
11	3	24 c.c.	HCl	8	70.0

The authors wish to acknowledge assistance from J. A. Leermakers and from several students in Chemistry 651 who carried out small-scale runs with a view to the use of this reaction in a standard, elementary course in organic chemistry. In this connection it is interesting to note that this as well as other preparations to be described are admirably suited for general laboratory instruction in organic chemistry, and have the special merit of starting with the very inexpensive furfural. We are also indebted to the Miner Laboratories of Chicago for liberal supplies of furfural.

#### SUMMARY

Conditions are described for the preparation in improved yields of furylacrylic acid from furfural by means of the Perkin condensation. Attention is directed to a miscellany of factors, particularly the repeated fusions of sodium acetate, which may be useful in other condensations of this type.

# A MONOGRAPH ON THE PROTOZOA OF THE LARGE INTESTINE OF THE HORSE

TA-SHII HSIUNG\*

*From the Department of Zoology, Iowa State College*

Accepted for publication April 25, 1930

## ACKNOWLEDGMENTS

While engaged in the preparation of this work the writer has had the great advantage of criticism and assistance from members of the Zoology Department, the Veterinary Pathology Department and the Veterinary Surgery Department. The writer takes pleasure in expressing his indebtedness to Dr. E. R. Becker, under whose direction the work was carried on, for his valuable criticism and helpful suggestions. He is grateful also to Dr. C. J. Drake for his kind interest and encouragement in the work, to Dr. H. H. Knight for many advices and suggestions, and to Drs. E. A. Benbrook and W. A. Aitken for their permission to procure the materials from the horses for this study.

## INTRODUCTION

The present paper attempts to assemble under one cover the description and illustrations of those protozoa found in the large intestine of the horse. Although Buisson (1923) has made such compilations of the infusoria of the mammals, the part which concerns the horse is quite inadequate, for he failed to include Gassovsky's work (1919), which contains ten new genera and fifteen new species, and since his time many new species and some new genera have been described from the horse.

In the preparation of this work all published descriptions of members of this distinctive fauna have been carefully studied. The previously described types encountered in the materials were restudied in view of previous descriptions. Where the information previously given was adequate, it was incorporated into the present account. In cases where characters were either incorrectly stated or lacking, the proper corrections and supplemental details have been added. A number of new species also are described in this paper. On the other hand, certain names have been placed in the synonymy, and some forms have been renamed. And finally, the ensemble of species has been treated systematically. This has resulted in the erection of one new family.

The main emphasis of this work has been placed upon the ciliates, as that is the group involving the largest number of species and the greatest

---

\*A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, major subject Zoology.



variety in respect to genera. They may be regarded as a distinct fauna of the horse.

#### REVIEW OF LITERATURE

The literature upon this subject is not so extensive as might be supposed, although it covers a period of eighty-seven years. The first information regarding the presence of protozoa in the large intestine of the horse was given by Gruby and Delafond (1843) under the title "Recherches sur des animalcules se développant en grand nombre dans l'estomac et dans les intestins, pendant la digestion des animaux herbivores et carnivores." In this communication they presented a short general account of the parasitic protozoa of the intestines of the dog, the pig, the horse and of the stomach of the ox. They enumerated seven species from the horse and described them briefly. Unfortunately, their descriptions were not accurate and, furthermore, they were not accompanied by illustrations. These facts make it impossible for the later workers to identify the protozoa which they observed. Yet this work is still important because of the early date at which it was done and because it did open up a new field for investigation.

The next information was given by Colin (1854) in his "Traité de physiologie comparée des animaux." Unfortunately the first edition of this book is not available to the writer. In the second edition (1871) he declared that in the cecum and colon of the horse there occurred eight to ten species of protozoa. He produced fifteen drawings from which the genera *Cycloposthium*, *Blepharocorys* and *Paraisotricha* can be recognized.

In 1869 Weiss referred to the discussion of Gruby and Delafond. He gave also a sketchy drawing of *Cycloposthium*.

In 1890 Fiorentini published two short papers dealing with ciliates which he found in the cecum and colon of the horse. He figured and described fourteen new species. He was apparently the first to name any of these protozoa and to classify them. To the genus *Diplodinium* he added two new species,—*Diplodinium uncinatum* and *Diplodinium unifasciculatum*. To the genus *Entodinium* he also added two new species,—*Entodinium valvatum* and *Entodinium bipalmatum*. But the descriptions of these new species were not in accord with the characteristics of the genera *Diplodinium* and *Entodinium* respectively, as laid down by former workers. Consequently, they were removed to new genera by later workers. In the genus *Paraisotricha* he included six heterogeneous species, some of which were undoubtedly synonyms due to inaccurate observations and some unquestionably should be removed from the genus. In general his drawings were crude and sometimes imaginary and his descriptions were far too brief.

The next work of importance was that of Bundle (1895) who published a rather voluminous account of his investigations entitled "Ciliate Infusorien im Coecum des Pferdes". In this paper he founded the genus *Cycloposthium* to which he assigned *Entodinium bipalmatum* Fiorentini. To the new genus *Blepharocorys* he referred both the *Entodinium valvatum* Fiorentini and also *Diplodinium uncinatum* Fiorentini. He did not discuss *Diplodinium unifasciculatum* Fiorentini. He added a new species *Blepharocorys jubata*, to the genus *Blepharocorys*. Besides redescribing the

genera, *Didesmis* Fiorentini and *Paraisotricha* Fiorentini, he added to the latter a new species, *Paraisotricha truncata*, which was unfortunately a synonym of *P. colpoidea*. He erected three more new genera, *Blepharoprosthium*, *Blepharosphaera* and *Blepharocodon*, in each of which he placed a new species. He also found a new ciliate, *Buetschlia postciliata*, which he referred to the genus *Buetschlia* described only from the stomachs of cattle before that time. Although he made many mistakes, his descriptions in general were clear and his drawings fairly good, but misleading.

In 1914 Sharp published a wonderful work on the minute morphology of *Diplodinium ecaudatum*. His discovery of the neuromotor apparatus and his descriptions of other structural details of *Diplodinium ecaudatum* stirred up the entire biological world and gave impetus for further researches along that line. In this work he also listed the ciliates described from the large intestine of the horse up to that time. Unfortunately, he referred *Diplodinium unifasciculatum* Fiorentini to the genus *Blepharocorys* Bundle.

In 1915 Schumacher worked on *Blepharocorys equi* and gave an excellent description of the morphology of that tiny ciliate from the cecum of the horse. It appears to the writer that this *Blepharocorys equi* is really a synonym of *Blepharocorys uncinata*.

In 1917 Cunha published a short note concerning the presence of *Balantidium* in the horse. He stated that this organism resembled *Balantidium coli* found in man, and he also pointed out the fact that the ciliates which commonly inhabit the cecum of the horse were not found together with this *Balantidium*.

In 1919 Gassovsky published a short paper, "On the microfauna of the intestine of the horse". In this work he erected ten new genera and fifteen new species. He transferred *Paraisotricha ovalis* Fiorentini to his new genus *Holophryoides* and *Diplodinium unifasciculatum* Fiorentini to his new genus *Tetratoxum*. Most of his drawings were simple and neat, but some of them were inaccurate. His descriptions were rather brief.

Fantham (1921) in his article "Some parasitic protozoa found in South Africa" reported the presence of *Endamoeba*, *Trichomonas* and *Giardia* in the large intestine of the horse. Only a few measurements and a short description were given for each of them.

In 1923 Buisson published two works, the one the "Thèse de Médecine" and the other an article entitled "Sur quelques infusoires nouveaux ou parconnus parasites des mammifères". In both these works he compiled a list of the ciliates found in the large intestine of the horse and gave a very brief description of each.

In 1925 Jameson described a new ciliate, *Charon ventriculi*, from the stomachs of ruminants. He stated that this ciliate was closely related to *Blepharocorys* found only in the large intestine of the horse. It is really fortunate that the writer also found on several occasions ciliates that resembled *Charon ventriculi* closely. By the cross infection experiments and by the critical microscopic examination it has been proved that it is a separate species. The writer had the privilege to name it *Charon equi*.

In 1928 Cunha and Muniz found a new ciliate which closely resembled *Buetschlia postciliata* Bundle in the cecum of *Tapirus americanus*. They

erected for this new species a new genus, *Bundleia* to which they also transferred *Buetschlia postciliata* Bundle.

In 1928 Strelkow published an excellent monograph on the genus *Cycloposthium*. Besides describing two new species, he redescribed *Cycloposthium bipalmatum* and separated *Cycloposthium edentatum* from *Cycloposthium dentiferum* Gassovsky, 1919. In the next year he again published an excellent paper on the morphology of *Cycloposthium*.

In 1928 the writer described two new species of *Suctorina* from the large intestine of the horse. It is really remarkable that *Suctorina* can be found in the digestive tract of a mammal. Since 1928 the writer has published descriptions of several more new species of ciliates from the large intestine of the horse.

In 1929 Dogiel published an excellent paper on the "concretion vacuole" of ciliates found in the large intestine of the horse. He regarded this structure as an organelle comparable to the statocysts of metazoan invertebrates.

#### TECHNIQUE

The material used in this work was obtained from horses that were sacrificed for the operative surgery and the postmortem technique classes of the Division of Veterinary Medicine of the Iowa State College. In every case extreme care was taken to avoid any fall in the temperature of the intestinal fluid in the interval between killing the horse and studying the living material. The protozoa were kept alive either in a Freas electric incubator or in a thermo-vacuum jug containing warm water of the desired temperature.

The living protozoa were studied on an electric warming stage. To avoid evaporation of the fluid, the cover-glass was sealed to the slide with vaseline. In this way the animals have been kept alive for several hours.

When the fluid to be studied was brought to the laboratory, a portion of it was either immediately fixed in Schaudinn's fluid or in two volumes of 10 per cent formalin. Sometimes wet-film slides were made and stained with iron-hematoxylin for the detailed studies of amoebae and flagellates. Permanent slides were made also from the fixed materials by staining them with iron-hematoxylin. The material fixed in formalin might be studied without further treatment, but staining with acid methyl green always made the nuclei more prominent.

#### Comments on Table 1

It should be noted that horse No. 4, which had been treated for worms not long before its sacrifice, only *Cycloposthium bipalmatum*, *Blepharocorys jubata* and flagellates were observed in the cecal material. Horse No. 5 had also been treated for bots, and only *Cycloposthium bipalmatum* and flagellates were observed in the cecal material and only *Cycloposthium bipalmatum*, *Blepharocorys curvigula*, *Blepharocorys angusta* and flagellates were observed in the colic material. Horse No. 16 underwent post-mortem examination about ten hours after its death, but many species of protozoa were found still alive.



TABLE 1. Showing the incidence of various species of protozoa found in the cecum, colon and feces of horses.

TABLE 1. Showing the incidence of various species of protozoa found in the cecum, colon and feces of horses.																																															Total								
Name of protozoa	Designation of horse																																														C	L	F						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46									
	6	1																																																					
<i>Endamoeba gedoelsti</i>		C	C				C								C				L	C			C										C		C		C	C	C			C					8	1							
<i>Oikomonas equi</i>																										CL	CL	C		C		C	C	C	C								L					14	4	2					
<i>Trichomonas equi</i>				C	CL			F			F				C		C			C					CL	CL	C		C			C		C	C	C						L						7	5						
<i>Callimastix equi</i>																C					CL		C	L	CL	CL		C				C				C	C	C	C	C	C	C			C			16	6	2					
<i>Didesmis ovalis</i>		C							F				C			CL	C			L		F		L	L	CL	C				C	C			C		C		C				C					8	3	4					
<i>D. quadrata</i>						CL				F	F											F	F	L						CL		C	C															2							
<i>D. spiralis</i>												C	C													C								C	C	C	C		C			L						13	4						
<i>Blepharoprosthium pireum</i>			C			CL						L		C			C	C	L						C									C	C							C						9	2	1					
<i>Blepharosphaera intestinalis</i>												C		C		CL				C	C		F						C						C	C						C						4	2						
<i>B. ellipsoidalis</i>												C							L																														2						
<i>Blepharoonus cervicalis</i>												L														L																							1	1					
<i>B. benbrookii</i>												L										F																											3	2	4				
<i>Bundelia postciliata</i>								F	F			CL	C			CL						F	F																										5	2					
<i>Alloiozona trizona</i>						C						C		C									F				C		L	L				C											C				3	1	1				
<i>Polymorpha ampulla</i>												L										F																C											21	6	1				
<i>Parasitricha colpoidea</i>		C	C			CL	C	F				CL		C	C		C	C	CL	CL	CL				C			C	CL		C			C	C	C							C						8	1					
<i>P. beckeri</i>												C	C	C			C									C			L														C						31	3					
<i>P. minuta</i>	C	C	C			C	C					C	C	C	C	C	C	C	C	CL	C	C		C		C	L	C		C	C	C	C	C	C	C	C	C	C	C			C	C	C	L			21	4					
<i>Blepharocorys uncinata</i>		C	C			CL							C	C	C	C		C	CL	C												L	C													L				1	4				
<i>B. valvata</i>																L																																		22	4				
<i>B. jubata</i>	C	C		C		C							C	C	C	C	C	C	C	C	CL		C			CL		CL						C	C		C								L					12	4				
<i>B. curvigula</i>					L	L		F	F			L				L					L		F	F	L	L	L				L	L																		8	6				
<i>B. angusta</i>					L			F	F	F	F	L				L						F	F	L	L	L																									1				
<i>B. cardionucleata</i>																																																		3	4				
<i>Charon equi</i>						L		F		F												F	F	L		L																									38	8	1		
<i>Cycloposthium bipalmatum</i>	C	C	C	C	CL	CL	C			F		CL	C	C	C	C	C	C	CL	C	CL		C			C	CL	C	L		C			C			C	C		C	C								16	2					
<i>C. dentiferum</i>							C																																											11	2				
<i>C. edentatum</i>	C											CL	C		C	C					C	C							L	C			C																	24	4				
<i>C. scutigerum</i>	C						C					C	C	C		C	C	C	CL		C					C	CL	C	L	C	CL	C			C	C			C	C	C	C								3	1				
<i>C. affinae</i>														C													C	CL	C			C																			7	1			
<i>C. corrugatum</i>							C																																												3				
<i>Spiroditinium equi</i>										F			L				L					F										L																		3	2				
<i>Triadinium caudatum</i>													L				L					F																													3	1			
<i>T. galea</i>																						F	F				L																								2	2			
<i>T. minimum</i>																						F			L																										2	2			
<i>Tetratoxum unifasciculatum</i>										F			L																																						1				
<i>T. excavatum</i>																																																				1			
<i>T. parvum</i>												L											F		L																										3	2			
<i>Tripalmaria dogieli</i>										F			L																																						1	1			
<i>Cochliatoxum periactum</i>										F			L																																						2	2			
<i>Ditozum funinucleum</i>										F			L																																							6	8	3	
<i>Allantosoma intestinalis</i>						L		F			F		L				L																																		2				
<i>A. dicorniger</i>																C		C																																	9				
<i>A. brevicorniger</i>																																																							

Source of material: cecum = C, colon = L, and feces = F.





TABLE 2. *Showing the pH value of the intestinal fluid of twelve horses. (With colorimetric method.)*

No.	Cecum	Colon
27	7.0	
28	6.8	6.8
32	7.2	
35	6.8	
36	6.8	
37	7.0	
38	7.0	
39	7.0	
41		7.2
42	6.8	
45	7.0	
46		7.2

# MORPHOLOGY AND TAXONOMY

## 1. Subphylum: PLASMODROMA Doflein, 1901

### a. Class: RHIZOPODA von Siebold, 1845

#### Order: Amoebida Calkins, 1902

#### Family: ENDAMOEBIDAE Calkins, 1926

#### Genus: *ENDAMOEBA* Leidy, 1879

### *Endamoeba gedoelsti* mon. nov. (for *Amoeba intestinalis* Gedoelst, 1911) (Pl. I, Fig. 1)

Gedoelst (1911) in his work stated "*Amoeba intestinalis*: est un hôte normal de l'intestin du cheval, du porc, du chat, du dindon et probablement d'autres animaux". In 1920 Fantham found on two occasions a few amoebae in the colon of horses at Onderstepoort. He referred them to *Amoeba* (*Entamoeba*) *intestinalis* Gedoelst. Next year he found them in the cecum of the horses in very small numbers.

The writer also found on several occasions amoebae in the large intestine of the horse in small numbers. On one occasion the number of amoebae was large enough to permit the making of permanent preparations.

The size of this amoeba ranges from 6.5 to 12.5  $\mu$  by 6 to 11  $\mu$ . Its food vacuoles contain bacteria. The nucleus is similar to the *Endamoeba coli* type. The karyosome, surrounded by a halo, is eccentric in position.

Since the name proposed by Gedoelst was preoccupied by *Amoeba intestinalis* Blanchard, 1885, the writer suggests *Endamoeba gedoelsti* in honor of the first observer.

Habitat: cecum and colon.

Geographic distribution: Belgium, S. Africa, U. S. A.

### **Endamoeba equi** Fantham, 1921

Fantham (1921) observed another kind of amoeba in the feces of two horses showing signs of intestinal malaise. It has the appearance of *Endamoeba histolytica* of man, except that it is rather larger and possesses an oval instead of a round nucleus. It ingests red blood corpuscles. The trophozoites were variable in size, but fully extended ones measured 40  $\mu$  to 50  $\mu$  by 23  $\mu$  to 29  $\mu$ , while rounded ones measured 28  $\mu$  to 35  $\mu$ . The cyst contained chromatoid bars, and four nuclei were present in the mature cyst. The diameter of cysts measured was 15  $\mu$ , 20  $\mu$ , and 24  $\mu$ .

Geographic distribution: S. Africa.

b. Class: MASTIGOPHORA Diesing, 1865

Subclass: ZOOMASTIGINA Doflein, 1916

Order: Protomonadina Blochmann, 1895

Family: MONADIDAE Kent, 1880

Genus: *OIKOMONAS* . Kent, 1880

### **Oikomonas equi, sp. nov.**

(Pl. I, Figs. 2-3)

Small flagellates each of which possesses a single flagellum were encountered by the writer in the material taken from the large intestine of the horse. The body-shape is variable but most of them show either a spherical or an oval shape. They are colorless and swim in a jerky manner. On one occasion this material was fixed in Schaudinn's fluid and stained with iron-hematoxylin. It possesses a spherical nucleus with a large central karyosome. It is situated at the anterior end. Near the surface of the anterior end of the body there is a blepharoplast, from which arises a single flagellum which is about four times as long as the body. The cytoplasm is filled with small dark staining granules. Its body length varies from 3.5 to 7  $\mu$  and its width varies from 3 to 5.5  $\mu$ . The length of the flagellum is about 20  $\mu$ .

Habitat: cecum.

Geographic distribution: U. S. A.

Family: TRICHOMONADIDAE Wenyon, 1926

Genus: *TRICHOMONAS* Donné, 1837

***Trichomonas equi* Fantham, 1921**

(Pl. I, Fig. 4)

Fantham (1921) has observed very rarely and in very small numbers *Trichomonas* in the intestine and feces of two horses. A fresh specimen measured 11  $\mu$  by 6  $\mu$ . On several occasions the writer also noted *Trichomonas* in the material collected from the large intestine of the horse. On one occasion some permanent preparations were made.

The flagellate seems to possess three anterior flagella and an undulating membrane. The axostyle is slender. The nucleus is anterior with a large karyosome. The measurements vary from 4 to 6.5  $\mu$  by 3 to 5  $\mu$ .

Habitat: cecum and colon.

Geographic distribution: S. Africa, U. S. A.

Family: CALLIMASTIGIDAE Fonseca, 1915

Genus: *CALLIMASTIX* Weissenberg, 1912

***Callimastix equi* Hsiung, 1929**

(Pl. I, Fig. 5)

Specific diagnosis.—*Callimastix*: The body is more or less kidney shaped and is rounded in cross-section. Its hilus is at the anterior third of the body. Just behind the hilus there is a clear, granule-free area, on the margin of which lie 12-15 basal granules. These basal granules give rise to 12-15 flagella. The rest of the cytoplasm is filled with deeply staining granules. The nucleus is 3  $\mu$  in diameter and shows a large karyosome. It is situated near the center of the body. The length of this flagellate ranges from 12-18  $\mu$ , with a mean of 14.4  $\mu$ . The width ranges from 7-10  $\mu$ , with a mean of 8.16  $\mu$ . The length of the flagella ranges from 25-30  $\mu$ . In living specimens these flagella are united and function as a unit.

This flagellate resembles closely *Callimastix frontalis* Braune, which is usually found in the stomachs of cattle, sheep and goats. It differs, however, from *C. frontalis* morphologically by its large size and the central location of its nucleus. Physiologically, it is found in the large intestine of the horse.

Habitat: cecum and colon.

Geographic distribution: U. S. A.

Order: Polymastigina Blochmann, 1895

Family: DISTOMATIDAE Senn, 1900

Genus: *GIARDIA* K nstler, 1882



**Giardia equi** Fantham, 1921

Fantham (1921) found *Giardia* in post-mortem material from the large colon of the horse which died in the district of Pretoria. The parasites were extremely few in number and observations were limited to the fresh state. The trophozoite gave measurements of 20  $\mu$  by 10  $\mu$ .

The writer has never been able to find *Giardia* in the large intestine of the horse. Its appearance there may have been due to migration after the death of the host.

Habitat: colon.

Geographic distribution: S. Africa.

Subphylum: **CILIOPHORA** Doflein, 1901

Phylum diagnosis.—Bearing cilia during entire or part of the life history; nuclear elements organized into larger macro-nucleus and smaller micro-nucleus.

Class 1: **CILIATA** Perty, 1852

Class diagnosis.—Cilia present throughout the life of the organism.

*Key to the Orders of Ciliata*

- |  |                       |
|--|-----------------------|
| 1. Body with adoral zone of membranelles ..... | 2                     |
| Body without adoral zone of membranelles ..... | <i>Holotrichida</i>   |
| 2. Body covered with cilia .....               | <i>Heterotrichida</i> |
| Cilia on body absent or much reduced .....     | <i>Oligotrichida</i>  |

Order: **Holotrichida** Delage and Hérourard, 1896

Order diagnosis.—A definite cytostome may or may not be present; cilia are more or less even length.

Suborder: *Stomatea* Wenyon, 1926

Suborder diagnosis.—*Holotrichida* which are provided with a cytostome.

*Key to Sections of Stomatea*

- |  |                      |
|--|----------------------|
| 1. Mouth usually closed; oral membranes absent ..... | <i>Gymnostomata</i>  |
| Mouth usually opened; oral membranes present .....   | <i>Trichostomata</i> |

Section 1: **GYMNOSTOMATA** Buetschli, 1889

Section diagnosis.—Cytostome usually closed, unprovided with cilia or membranes; esophagus, if present, naked or supported by rod-apparatus; peristome usually absent.

Family (1): **BUETSCHLIIDAE** Poche, 1913

Family diagnosis.—Round cytostome at the anterior end, at the posterior end a cypopyge. Body either uniformly covered with cilia or limited to certain areas. Concretion vacuole containing a mass of granules near anterior end. One or more contractile vacuoles.

Type-genus: *Buetschlia* Schuberg, 1888.

To this family the writer adds the following genera from the horse: *Didesmis* Fiorentini, 1890; *Blepharoprosthium* Bundle, 1895; *Blepharosphaera* Bundle, 1895; *Holophryoides* Gassovsky, 1919; *Blepharozoum* Gassovsky, 1919; *Prorodonopsis* Gassovsky, 1919; *Paraisotrichopsis* Gassovsky, 1919; *Blepharoconus* Gassovsky, 1919; *Bundleia* Cunha et Muniz, 1928; *Alloiozona* Hsiung, 1930; *Polymorpha* Dogiel, 1929 and *Ampullacula* gen. nov.

Hickson (1903) referred the genera *Blepharocodon*, *Blepharoprosthium* and *Blepharosphaera* to the family *Prorotrichina*, *Buetschli*, 1889. Poche (1913) made a new name, family *Buetschliidae*, to include *Buetschlia* Schuberg as well as the other three genera described by Bundle. *Didesmis* was included in the family *Ophryoscolicina* Stein, 1858, by Hickson, although he admitted that it should be separated from that family. Poche included the genus *Didesmis* in his new family *Cycloposthiidae*. It was Cunha (1919) who transferred *Didesmis* to the family *Buetschliidae*. Because of the absence of a concretion vacuole inside the cytoplasm, the genus *Blepharocodon* Bundle, 1895 should be removed from the family *Buetschliidae*.

# Key to the Genera of *Buetschliidae*

1. Body covered with fine cilia, neck\*covered with thick and long cilia ..... *Ampullacula*  
 Body uniformly covered with cilia ..... 2  
 Body cilia limited to certain areas ..... 5
2. Presence of a spiral groove ..... *Paraisotrichopsis*  
 Absence of a spiral groove ..... 3
3. Wide anterior peristome; contractile vacuole single .... *Holophryoides*  
 Oral aperture bent to one side; contractile vacuole two or more 4
4. Body length less than 100  $\mu$ ; macronucleus sausage-shaped..... *Prorodonopsis*  
 Body length more than 200  $\mu$ ; macronucleus bean-shaped. *Blepharozoum*
5. Body cilia arranged in two zones ..... 6
6. Body cilia limited to both anterior and posterior extremities .. 7  
 Body cilia limited to at least anterior half of the body and posterior extremity ..... 8
7. Wide anterior peristome; posterior cilia as numerous as anterior cilia ..... *Didesmis*  
 Small anterior peristome; a few posterior cilia ..... *Bundleia*
8. Body cilia covered more than half of the body; only a narrow zone between the two ciliary areas ..... *Blepharosphaera*  
 Body cilia covered less than half of the body; wide zone between the two ciliary areas ..... 9
9. Rod-like or fibrous structures present inside the cytoplasm.. *Blepharoconus*  
 No such kind of structures present inside the cytoplasm ..... 10
10. Body length more than 50 $\mu$ ; macronucleus sausage-shaped.. *Blepharoprosthium*  
 Body length less than 40  $\mu$ ; macronucleus disc-shaped .... *Polymorpha*

Genus: *DIDESMIS* Fiorentini, 1890

Generic diagnosis.—Buetschliidae: cytostome broad; definite neck posterior to the cytostome; cilia at both anterior and posterior ends.

Type-species: *Didesmis quadrata* Fiorentini, 1890.

*Key to Species of Didesmis*

1. Without dorsal groove ..... *D. ovalis*  
     With dorsal groove ..... 2
2. Body and groove spiral ..... *D. spiralis*  
     Body and groove not spiral ..... *D. quadrata*

1. *Didesmis ovalis* Fiorentini, 1890

(Pl. I, Fig. 6)

Specific diagnosis.—*Didesmis*: Seen from the dorsal side this protozoon is oval or rectangular in shape, the anterior end being blunt and the posterior end tapering. The body is rigid, noncontractile and about  $1\frac{1}{2}$  times as long as it is wide. It is widest at the middle of the body. Usually a slight depression just back of the anterior end forms a short neck. It is slightly flattened dorso-ventrally.

The anterior end is surrounded with long, fine cilia from the cytostome to the neck. The second ciliary zone surrounds the cytoppyge at the posterior end. The rest of the body is naked.

The ectoplasm is a thin and homogeneous layer, but considerably thicker at both ends. The endoplasm is granular.

The cytostome is situated at the middle of the anterior extremity of the body. The esophagus is short and funnel-shaped. The cytoppyge lies at the middle of the posterior extremity and it is connected by an anal tube.

The macronucleus is situated in the middle of the body just a little to the right. It is irregular oval in shape and distinctly granular. A small elipsoid and very refractive micronucleus is situated in a depression of the right surface of the macronucleus.

A concretion vacuole which contains numerous small refractive concretion granules lies near the anterior end of the macronucleus and a little to the right of it.

A contractile vacuole is situated on the right side in the posterior ventral part of the body. The second contractile vacuole as referred to by Bundle could not be confirmed. Reproduction is by transverse fission.

The measurements of 49 specimens yields the following results:

	Length	Width
Range .....	34 - 55 $\mu$	27 - 40 $\mu$
Mean .....	46.45 $\pm$ 0.47 $\mu$	31.70 $\pm$ 0.20 $\mu$
Standard deviation .....	4.96 $\mu$	2.10 $\mu$
Coefficient of variation ...	10.67 %	6.62 %

Habitat: cecum and colon.

Geographic distribution: Italy, Russia, U. S. A. and Germany.

## 2. *Didesmis quadrata* Fiorentini, 1890

(Pl. I, Fig. 7)

**Specific diagnosis.**—*Didesmis*: In general shape and texture this protozoon is similar to the preceding species except for the presence of a deep, wide, highly refractive groove on the dorsal surface. It is about  $1\frac{1}{2}$  times as long as it is wide. It is widest at the middle. A neck is also present and it is also dorso-ventrally flattened. This groove runs longitudinally on the dorsal surface parallel to the long axis of the body. Its anterior end extends near to the cytostome while its posterior end extends to the vicinity of the anal tube. Numerous fine, transverse striation can usually be seen in the groove. The ventral surface is slightly concave and there is not any sharp ridge as is referred to by Bundle. The two ciliary zones are also the same as in the preceding species.

The cytostome is situated at the middle of the anterior extremity of the body. The esophagus is short and funnel-shaped. It is surrounded with long and fine cilia. The cytopye lies at the middle of the posterior extremity. It is connected by an anal tube. The anal area is also surrounded by cilia.

The macronucleus is spindle shaped and granular and is situated at the middle of the body under the groove, a little to the right side. A small ellipsoidal micronucleus is situated in a depression at the right side of the macronucleus.

A concretion vacuole which contains numerous small refractive concretion granules lies near to the anterior end of the macronucleus and a little to the right of it. It lies close to the ventral side of the body.

A contractile vacuole is situated on the right side in the posterior ventral part of the body. The second contractile vacuole mentioned by Bundle could not be confirmed. Reproduction is by transverse fission.

The measurements of 25 specimens yielded the following results:

	Length	Width
Range .....	50 - 90 $\mu$	33 - 68 $\mu$
Mean .....	$68.84 \pm 1.33 \mu$	$48.44 \pm 1.08 \mu$
Standard deviation .....	9.93 $\mu$	8.10 $\mu$
Coefficient of variation ...	14.21 %	16.72 %

**Habitat:** cecum and colon.

**Geographical distribution:** Italy, Germany, Russia, S. Africa, Brazil, U. S. A.

## 3. *Didesmis spiralis* Hsiung, 1929

(Pl. I, Fig. 8)

**Specific diagnosis.**—*Didesmis*: In general structure and texture this protozoon is similar to *D. quadrata* Fiorentini, except that it is spirally shaped. The dorsal groove runs slightly diagonally to the long axis of the body with its anterior end inclined to the right side and its posterior end inclined to the left side. Anteriorly it reaches the neck and posteriorly it extends to the vicinity of the anal tube. The ventral side of the body is slightly concave.



The ciliary zones as in the other species are located at the anterior and posterior extremities. The rest of the body is naked.

The cytostome is triangular in shape and is situated on the ventral side of the anterior end of the body. The esophagus is short and more or less funnel-shaped. The cytopyge lies at the posterior end of the body. It is connected with a comparatively long anal tube.

The macronucleus is situated under the groove in the middle and in the direction of the long axis of the body a little to the right side. It is spindle-shaped and is distinctly granular. The small subspherical micronucleus is situated in a depression on the right ventral surface of the macronucleus.

A concretion vacuole which contains less than ten refractive concretion granules lies near the anterior end of the macronucleus and a little to the right of it. A contractile vacuole is usually situated in the posterior ventral part of the body. Reproduction is by transverse fission.

The measurements of 27 specimens yield the following results:

	Length	Width
Range .....	60 - 94 $\mu$	38 - 54 $\mu$
Mean .....	75.96 $\pm$ 1.07 $\mu$	43.1 $\pm$ 0.51 $\mu$
Standard deviation .....	8.22 $\mu$	3.97 $\mu$
Coefficient of variation ...	10.83 %	9.21 %

Habitat: cecum.

Geographic distribution: U. S. A.

Genus: *BLEPHAROPROSTHIUM* Bundle, 1895

Generic diagnosis.—Buetschliidae: Anterior half of body contractile and ciliated ;small tuft of anal cilia; macronucleus sausage-shaped.

Type-species: *Blepharoprosthium pireum* Bundle, 1895.

### ***Blepharoprosthium pireum* Bundle, 1895**

(Pl. I, Figs. 9-10)

Synonym: *Blepharoprosthium musculus* Dogiel, 1929.

Specific diagnosis.—*Blepharoprosthium*: The body is pearshaped. It tapers toward the anterior end, while the posterior half is hemispherical. Its anterior end is contractile and ciliated. There is also a small tuft of anal cilia. The space between these two ciliary zones is small.

The cytostome is anterior and is followed by a funnel-shaped esophagus. The cytopyge is at the posterior end.

The ectoplasm is a homogeneous layer, while the endoplasm is coarsely granular and is filled with large granules and particles of food. The anterior is always clear. The writer has seen *Blepharocorys uncinata* inside the cytoplasm on two occasions. When the organism swims, the body shape is cylindrical and the anterior end is truncated. When the anterior end is in contact with an object, the cytostome widens and the organism twists itself around.

The macronucleus is sausage-shaped and is not constant in its position. A subspherical micronucleus lies at the middle of the inner curvature of the macronucleus.

A concretion vacuole which contains numerous granules is situated at the anterior half of the body close to the surface. The ectoplasm slightly bulges above it. There is a contractile vacuole at the posterior end. Reproduction is by transverse fission:

Measurements of 49 specimens yielded the following results:

	Length	Width
Range .....	54 - 86 $\mu$	34 - 52 $\mu$
Mean .....	70.67 $\pm$ 0.82 $\mu$	44.61 $\pm$ 0.41 $\mu$
Standard deviation .....	8.65 $\mu$	4.35 $\mu$
Coefficient of variation ...	12.24 %	9.75 %

Habitat: cecum and colon.

Geographic distribution: Germany, Russia, U. S. A.

Genus: *BLEPHAROSPHAERA* Bundle, 1895

Generic diagnosis.—Buetschliidae: Body spherical or ellipsoidal; uniformly covered with cilia except at the posterior part; anal cilia present.

Type-species: *Blepharosphaera intestinalis* Bundle, 1895.

*Key to the Species of Blepharosphaera*

- 1. Body spherical ..... *B. intestinalis*
- Body ellipsoidal ..... *B. ellipsoidalis*

1. *Blepharosphaera intestinalis* Bundle, 1895

(Pl. I, Fig. 11)

Specific diagnosis.—*Blepharosphaera*: Body is spherical. Its form is constant. It is uniformly covered with cilia except at the posterior part. At the posterior extremity, there are usually a few anal cilia.

The ectoplasm is a homogeneous layer. The endoplasm is granular and contains food particles. The anterior end is usually much more clear. The cytostome is circular and is surrounded by dense cilia. It leads into a short funnel-shaped esophagus. The cytopyge is indistinct.

The macronucleus is not constant in its position. It is a thick ellipsoidal disc. A small subspherical micronucleus can usually be found at the end of the macronucleus.

A concretion vacuole containing numerous granules is located at the anterior part of the body just anterior to the middle. At the same side of the posterior part of the body there is a large contractile vacuole. Dividing forms have not been observed.

Measurements of 25 specimens yield the following results:

	Length	Width
Range .....	38 - 74 $\mu$	38 - 74 $\mu$
Mean .....	48.36 $\mu$	48.36 $\mu$

Habitat: cecum and colon.

Geographic distribution: Germany, U. S. A.

## 2. *Blepharosphaera ellipsoidalis* sp. nov.

(Pl. I, Fig. 12)

Specific diagnosis.—*Blepharosphaera*: Body is ellipsoidal. The other characters are almost identical with those of *B. intestinalis*. It is also uniformly covered with cilia except at the posterior part. The anal cilia are also present. The cytostome is circular and the esophagus is longer and triangular. The cytopye is distinct.

The macronucleus is not constant in its position. It is sausage-shaped. The small subspherical micronucleus is situated in the depression at the end of the macronucleus.

The concretion vacuole is located nearer to the anterior end. The small contractile vacuole is located near the cytopye. Dividing forms have not been observed.

Measurements of 25 specimens yield the following results:

	Length	Width
Range .....	34 - 65 $\mu$	27 - 49 $\mu$
Mean .....	50.52 $\mu$	39.36 $\mu$

Habitat: cecum and colon.

Geographic distribution: U. S. A.

Genus: *HOLOPHRYOIDES* Gassovsky, 1919

*Holophryoides ovalis* (Fiorentini, 1890) Gassovsky, 1919

(Pl. I, Fig. 13)

Synonym.—*Paraisotricha ovalis* Fiorentini, 1890; *Paraisotricha triangularis* Fiorentini, 1890.

Specific diagnosis.—*Holophryoides*: "Body oviform or elliptical, covered entirely with dense cilia. Oral aperture at the end of a coniform projection in a wide peristome. Cytopye near posterior end of the body. A short proctodeum is present. Ectoplasm forms an accumulation at the anterior part of the body. Contractile vacuole—single, in the posterior end of the body. In its anterior third there is a 'vacuole with concretions'. Macronucleus elliptical. In a depression at one of its ends lies the micronucleus. Measurements: 95-140  $\mu$  x 65-90  $\mu$ . This was referred by Fiorentini (1890) to the genus *Paraisotricha*."

Habitat: cecum and colon.

Geographic distribution: Italy, Russia.

Genus: *BLEPHAROZOOM* Gassovsky, 1919

**Blepharozoum zonatum** Gassovsky, 1919

(Pl. I, Fig. 14)

Specific diagnosis.—Buetschliidae: “Body oviform, covered with long and fine cilia. When the infusor moves the cilia adhere nearer to the surface of the body in two places of the body forming two constrictions, so that the whole ciliary area is divided into three zones. The oral aperture is situated near the apex of the wide end of the body. The cytopyge—near its posterior end. Contractile vacuoles—2-4; one in the posterior end of the body. In its anterior end is situated a ‘vacuole with concretions’. Macronucleus bean-shaped. In the middle of its flat side lies the micronucleus. Measurements:  $230-245\ \mu \times 115-122\ \mu$ .”

Habitat: cecum.

Geographic distribution: Russia.

Genus: *PRORODONOPSIS* Gassovsky, 1919

**Prorodonopsis coli** Gassovsky, 1919

(Pl. I, Fig. 15)

Specific diagnosis.—Buetschliidae: “Body oviform. Anterior end with oral aperture somewhat bent to the side. The entire body-surface is covered with cilia. The endoplasm contains: ‘vacuole with concretions’—in the anterior region, a sausage-shaped macronucleus—in the posterior half, and 2-3 contractile vacuoles—in the posterior end. Measurements:  $55-67\ \mu \times 38-45\ \mu$ .”

Habitat: colon.

Geographic distribution: Russia.

Genus: *PARAISOTRICHOPSIS* Gassovsky, 1919

**Paraisotrichopsis composita** Gassovsky, 1919

(Pl. I, Fig. 16)

Specific diagnosis.—Buetschliidae: “Body elliptical, covered with cilia, except the spiral groove. The latter begins from the oral aperture in the anterior part of the ventral side of the body, passes along the dorsal surface and terminates at the posterior end of the body. Owing to the groove the latter seems to be composed of two halves displaced with regard to each other in the direction of the long axis. In the anterior part of the endoplasm lies the ‘vacuole with concretions’, the elongated macronucleus and micronucleus, in the posterior part—the contractile vacuole. Measurements:  $43-56\ \mu \times 31-40\ \mu$ .”

Geographic distribution: Russia.

Genus: *BLEPHAROCONUS* Gassovsky, 1919

Generic diagnosis.—Buetschliidae: Small terminal peristome. Anterior and posterior ends of the body covered with cilia. Esophagus guarded by rod-like structure.

Type-species: *Blepharoconus hemiciatus* Gassovsky, 1919.



*Key to the Species of Blepharoconus*

1. Body cone-shaped; body length more than  $83\ \mu$  ..... *B. hemiciiliatus*  
     Body ovoid or ellipsoid; body length less than  $83\ \mu$  ..... 2
2. Body length more than  $50\ \mu$ ; three contractile vacuoles .... *B. cervicalis*  
     Body length less than  $40\ \mu$ ; a single contractile vacuole ... *B. benbrooki*

**1. *Blepharoconus hemiciiliatus* Gassovsky, 1919**

(Pl. I, Fig. 17)

Specific diagnosis.—*Blepharoconus*: "Body conical. Cilia covering only the anterior part of the body. Besides a small number of cilia surrounded the ectopyge with the proctodeum at the posterior end. The cytoplasm is transparent. Contractile vacuole in the number of three; one—posteriorly to the 'vacuole with concretions,' two others—on the opposite side of the body. The macronucleus is nearly spherical. At one of its ends lies the micronucleus. Measurements:  $83\text{--}135 \times 45\text{--}65\ \mu$ ."

Habitat: colon.

Geographic distribution: Russia.

**2. *Blepharoconus cervicalis* Hsiung, 1930**

(Pl. I, Fig. 18)

Specific diagnosis.—*Blepharoconus*: The body shape is ovoid, with blunt anterior and rounded posterior end which is slightly displaced to one side. Usually a slight groove which circles the part just back of the anterior end forms a short neck. The anterior half of the body is covered with long, fine cilia. A bundle of cilia is also found around the ectopyge at the posterior part of the body. The rest of the body is naked.

The ectoplasm is homogeneous. The endoplasm is coarsely granular, opaque, and contains particles of food. Just behind the neck the endoplasm is slightly transparent. There are many rod-like structures taking their origin at the anterior end inside the endoplasm. The peristome is located on a knob at the anterior end of the body. The ectopyge is located at the posterior end.

The macronucleus is indefinite in its position. It is more or less disc-shaped and distinctly granular. An ellipsoidal micronucleus is situated in a depression at the margin of the macronucleus.

An ellipsoidal concretion vacuole which contains a small number of granules is situated behind the neck. The ectoplasm slightly bulges above it. There are three contractile vacuoles; one is located just behind the concretion vacuole, and the other two on the opposite side. Reproduction is by transverse fission.

The measurements of 33 specimens yield the following results:

	Length	Width
Range .....	$56\text{--}83\ \mu$	$48\text{--}70\ \mu$
Mean .....	$70.33 \pm 0.80\ \mu$	$57.00 \pm 0.65\ \mu$
Standard deviation .....	$7.25\ \mu$	$5.81\ \mu$
Coefficient of variation ...	$10.30\ \%$	$10.17\ \%$

Habitat: colon.

Geographic distribution: U. S. A.

3. *Blepharoconus benbrooki* sp. nov.

(Pl. I, Fig. 19)

Specific diagnosis.—*Blepharoconus*: The body shape varies from ovoid to ellipsoid. The anterior end forms a knob and the posterior end is rounded. The anterior end of the body is covered with long, fine cilia and the posterior end is also covered with a smaller bundle of long, fine anal cilia. The rest of the body is naked.

The ectoplasm is homogeneous and the endoplasm is granular and contains small particles of food. There are fibrous structures inside the endoplasm. They take their origin at the anterior end and converge posteriorly a little to the side. The cytopyge which is surrounded by the anal cilia is located at the posterior end.

The macronucleus is indefinite in its position. It is a thick disc and distinctly granular. An ellipsoidal micronucleus is situated in a depression at the margin of the macronucleus.

There is a large ellipsoidal concretion vacuole which contains a large number of granules situated at the anterior end with one of its ends placed near to the surface. The ectoplasm slightly bulges above it. There is a large contractile vacuole at the posterior end of the body near to the anal opening. Dividing forms have not been observed.

Measurements of 25 specimens yield the following results:

	Length	Width
Range .....	21 - 37 $\mu$	17-26 $\mu$
Mean .....	28.40 $\pm$ 0.62 $\mu$	20.92 $\pm$ 0.21 $\mu$
Standard deviation .....	4.63 $\mu$	2.17 $\mu$
Coefficient of variation ...	16.32 %	10.37 %

Habitat: colon.

Geographic distribution: U. S. A.

The writer takes great pleasure in naming this species after Dr. E. A. Benbrook of the Veterinary Pathology Department.

Genus: *BUNDLEIA* Cunha et Muniz, 1928

Generic diagnosis.—*Buetschliidae*: Cilia at anterior and posterior ends; cytostome small.

Type-species: *Bundelia postcilia* (Bundle, 1895) Cunha et Muniz, 1928.

*Bundleia postciliata* (Bundle, 1895) Cunha et Muniz, 1928

(Pl. I, Fig. 20)

Synonym: *Buetschlia postciliata* Bundle, 1895.

Specific diagnosis.—*Bundleia*: Body is slightly flattened dorso-ventrally. The lateral surfaces are slightly convex and converge gently toward the posterior end. At the anterior part it tapers sharply and is finally truncated at the anterior extremity. Its posterior extremity is also truncated. Both anterior and posterior ends are provided with long, fine cilia. The rest of the body is naked.

The ectoplasm is homogeneous and thickened and its endoplasm is coarsely granular. The peristome is located at the anterior end and is followed by a short funnel-shaped esophagus. The cytopyge is located at the posterior extremity and is followed by an anal tube.

The macronucleus is indefinite in its position. It is ellipsoidal in shape and distinctly granular. A small subspherical micronucleus is situated at the side of the macronucleus.

A small concretion vacuole is found at the right side near to the anterior end. It contains a small number of concretion granules. A small contractile vacuole is usually found at the left corner of the posterior end. Reproduction is by transverse division.

The measurements of 18 specimens yield the following results:

	Length	Width
Range .....	30 - 56 $\mu$	17 - 32 $\mu$
Mean .....	41.88 $\mu$	25.55 $\mu$

Habitat: cecum and colon.

Geographic distribution: Germany, S. Africa, U. S. A., Brazil.

#### Genus: *ALLOIOZONA* Hsiung, 1930

Generic diagnosis.—Buetschliidae: Body cilia divided into three zones; areas between these ciliary zones are naked.

Type-species: *Alloiozona trizona* Hsiung, 1930; only species of the genus.

#### *Alloiozona trizona* Hsiung, 1930 (Pl. II, Figs. 21-22)

Specific diagnosis.—*Alloiozona*: This protozoon is ovoid with both ends rounded. The posterior end is more tapering than the anterior end. The body is less than twice as long as it is wide. It is widest at the middle. The body cilia are arranged in definite striations in three distinct zones,—the anterior, the middle and the posterior. The areas between these three ciliary zones are naked.

The ectoplasm is a thin homogeneous layer except it is thicker above the concretion vacuole. The endoplasm is granular and contains large particles of food. The cytostome is at the anterior end which is surrounded by a shallow groove provided with short cilia. The esophagus is funnel-shaped. The cytopyge is situated on a knob at the posterior end and a short anal canal can also be seen.

The macronucleus is not constant in its position. It is more or less a thick disc and distinctly granular. A subspherical micronucleus is situated in a depression at the margin of the macronucleus.

A large concretion vacuole which contains numerous small, highly refractive concretion granules is situated near the surface at the anterior third of the body. A small contractile vacuole is usually found at the posterior end. Reproduction is by transverse fission. Specimens parasited by *Sphaerita* have also been observed.

Measurements of 36 specimens yield the following results:

	Length	Width
Range .....	50 - 90 $\mu$	30 - 60 $\mu$
Mean .....	$73.50 \pm 1.00 \mu$	$47.05 \pm 0.56 \mu$
Standard deviation .....	9.04 $\mu$	5.05 $\mu$
Coefficient of variation ...	12.29 %	11.58 %

Habitat: cecum and colon.

Geographic distribution: U. S. A.

Genus: *POLYMORPHA* Dogiel, 1929

Generic diagnosis.—Buetschliidae: Body flask-shaped; anterior part ciliated; small tuft of anal cilia; macronucleus disc-shaped.

Type-species: *Polymorpha ampulla* Dogiel, 1929.

***Polymorpha ampulla* Dogiel, 1929**

(Pl. II, Fig. 23)

Specific diagnosis.—*Polymorpha*: Body is flask-shaped. Its anterior end is ciliated and there is also a small tuft of anal cilia at the posterior end.

The ectoplasm is a homogeneous layer while the endoplasm is slightly granular and contains small food particles. The anterior end is clear and noncontractile. The cytostome is at the anterior end and is followed by a small triangular esophagus. The cypopyge is posterior.

The macronucleus is disc-shaped and is not constant in its position. A spherical micronucleus lies at its side. The concretion vacuole which contains only a few granules is situated in the anterior half of the body and it is more or less spindle-shaped. A contractile vacuole is situated at the posterior end.

Measurements of 25 specimens yield the following results:

	Length	Width
Range .....	22 - 36 $\mu$	13 - 21 $\mu$
Mean .....	$29.20 \pm 0.45 \mu$	$15.88 \pm 0.27 \mu$
Standard deviation .....	3.41 $\mu$	2.04 $\mu$
Coefficient of variation ...	11.67 %	12.84 %

Habitat: cecum and colon.

Geographic distribution: Russia, U. S. A.

Genus: *AMPULLACULA* gen. nov.

Generic diagnosis.—Buetschliidae: Body is covered with fine cilia, while neck is covered with thick and long cilia.

Type-species: (*Paraisotricha*) *ampulla* Fiorentini, 1890.



**Ampullacula ampulla** (Fiorentini, 1890)

(Pl. II, Fig. 24)

Synonym: *Paraisotricha ampulla* Fiorentini, 1890.

Specific diagnosis.—*Ampullacula*: “As a whole it presents the form of a flask and is covered with two very different kinds of cilia; that is, the body is covered with fine, almost imperceptible cilia, while the neck is covered with thick and long cilia; the neck ends in a very small peristome. One observes likewise an endoplasm as if made of balls, a nucleus, pigmented masses and the usual hyaline zone.”

Its measurements are: Length 110  $\mu$ ; width 40  $\mu$ .

Geographic distribution: Italy.

Since it deviates from the generic characters of *Paraisotricha*, it seems advisable to the writer to remove it from the genus. The new generic name, *Ampullacula*, is therefore proposed.

## SYSTEMATIC POSITION UNCERTAIN

Genus: *BLEPHAROCODON* Bundle, 1895**Blepharocodon appendiculatus** Bundle, 1895

(Pl. II, Fig. 25)

Specific diagnosis.—Body is bell-shaped. The oral end is broad and tapers toward the aboral end which is rounded. At the anterior end there is a tongue-shaped projection. The body is rigid, inelastic and non-contractile. Cilia are found only at the anterior end.

The ectoplasm is a thin homogeneous layer and the endoplasm is granular and contains food particles. The peristome occupies the whole anterior end is connected by an esophagus. A row of oral cilia circles around the peristome. No anal opening was observed.

The macronucleus is ovoid and coarsely granular and it is situated at the anterior end. No micronucleus was found. No contractile vacuole was observed. Dividing form was also not observed.

Measurements of five specimens yield the following results: mean length 35  $\mu$ , mean width 11  $\mu$ , mean thickness 10  $\mu$ .

Habitat: cecum.

Geographic distribution: Germany.

Since this form does not have a concretion vacuole, it should be removed from the family Buetschliidae, one of whose generic characters is the possession of the concretion vacuole.

Section 2: *TRICHOSTOMATA* Buetschli, 1889

Section diagnosis.—Cytostome permanently open, provided with cilia or membranes; esophagus, if present, ciliated or bearing membranes; peristome usually present.

*Key to the Families of Trichostomata*

1. Body uniformly covered with cilia; concretion vacuole present ..... *Paraisotrichidae*
- Body cilia limited to certain areas; concretion vacuole absent ..... *Blepharocoridae*

Family: **PARAISOTRICHIDAE** Cunha, 1916.

Family diagnosis.—*Trichostoma*: Body uniformly covered with cilia in slightly spiral longitudinal rows; a tuft of longer cilia at anterior end; cytostome just ventral to anterior concretion vacuole. Single contractile vacuole at posterior end.

Type-genus: *Paraisotricha* Fiorentini, 1890.

Hickson (1903) referred *Paraisotricha* Fiorentini to the family *Iso-trichina* Buetschli, 1887. Poche later referred it to the family *Isotrichidae* Schouteden, 1906. It was again Cunha (1917) who made a new family *Paraisotrichidae* for the genera *Paraisotricha* and *Blepharocorys*.

Genus: **PARAISOTRICHIA** Fiorentini, 1890

Generic diagnosis.—*Paraisotrichidae*: With the characters of the family.

Type-species: *Paraisotricha colpoidea* Fiorentini, 1890.

*Key to Species of Paraisotricha*

1. Only 11 ciliary striations on the whole body surface ..... *P. beckeri*
- More than 11 ciliary striations on the whole body surface .... 2
2. Body more than 70  $\mu$  in length ..... *P. colpoidea*
- Body less than 70  $\mu$  in length ..... *P. minuta*

1. ***Paraisotricha colpoidea*** Fiorentini, 1890

(Pl. II, Fig. 26)

Synonyms: *Paraisotricha oblonga* Fiorentini, 1890; *Paraisotricha truncata* Bundle, 1895; *Paraisotricha incisa* Fiorentini, 1890; *Paraisotricha magna* Dogiel, 1929.

Specific diagnosis.—*Paraisotricha*: The body is ovoid. It is somewhat elastic, and is about twice as long as it is wide. It is widest in the region just anterior to the middle of the body. The body is covered with fine cilia which are borne in grooves on tops of ridges. These cilia bearing ridges comprise 34 to 40 more or less spiral rows. These striations run spirally forward, turning toward the right, from the aboral pole where they have their points of origin about the cytopyge. Some of these striations stop at the ventral margin of the cytostome, but the remainder continue to the anterior end where the longer frontal cilia of the head region originate. These frontal cilia are just a trifle longer than the rest of the body cilia. It is not true that they are five times as long as the rest, as Bundle has stated.

The ectoplasm is homogeneous, while the endoplasm is granular and contains particles of food. The cytostome is a slanting slit with the right end more anterior on the ventral side of the anterior end of the body. Its ventral lip is guarded by a row of short cilia which extends to the right beyond the cytostome. The esophagus is long, funnel-shaped and is ciliated along its left lateral wall. The cytoppyge is a longitudinal slit located dorso-ventrally and a little to the left at the posterior end of the body. There is a short anal tube.

The macronucleus is a thick ellipsoidal disc and it is not constant in its position. It is distinctly granular. The small subspherical micronucleus is situated in a depression at the end of the disc-shaped macronucleus. No second micronucleus is observed.

A large concretion vacuole which contains numerous refractive concretion granules occupies nearly the whole anterior extremity of the body. A large contractile vacuole is situated in the posterior half of the body, a little to the right. There are no contractile vacuoles on the side of the esophagus. Reproduction is by transverse fission. Exconjugants have been observed. Specimens parasitized by *Sphaerita* have also been noted.

Measurements of 49 specimens yield the following results:

	Length	Width
Range .....	70 - 100 $\mu$	42 - 60 $\mu$
Mean .....	82.69 $\pm$ 0.75 $\mu$	48.55 $\pm$ 0.44 $\mu$
Standard deviation .....	7.88 $\mu$	4.62 $\mu$
Coefficient of variation ...	9.52 %	9.51 %

Habitat: cecum and colon.

Geographic distribution: Italy, Germany, Brazil, U. S. A., Russia.

Under certain conditions which it has not possible to explain at present the posterior part of the living specimen shrivels up to become triangular in shape. This peculiarity lead both Fiorentini and Bundle to recognize this deformed specimen as a species separate from *P. colpoidea*. The writer was fortunate enough to observe the transformation of specimen of *colpoidea*-type to that of *oblonga*-type, as well as the reverse. It is clear then that *P. oblonga* is just a synonym of *P. colpoidea*.

Under other occasions some of the specimens of *P. colpoidea* will rotate constantly in the same spot, finally the anterior part becomes twisted and the posterior extremity sunken in. At other times the same specimen will swim off in a normal way, the anterior end straightened out but the posterior end still in the truncated condition. The writer ventures to suggest that Bundle's *P. truncata* is just a truncated specimen of *P. colpoidea*.

The writer agrees with Bundle's contention that Fiorentini's *P. incisa* is just a broken specimen of *P. colpoidea*.

*P. triangularis* Fiorentini closely resembles *Holophryoides ovalis* except that its posterior end is triangular in shape. This change of shape may be due to the same condition that changes the shape of *P. colpoidea*. Therefore the writer considers that *P. triangularis* is a synonym of *H. ovalis*. So far the writer has not seen any specimen that resembles *P. ampulla* in over 40 horses that he has examined. If this species is really what Fiorentini has figured and described, it should be removed from the

genus *Paraisotricha* and a new genus should be erected for the reception of this species. The writer has already suggested the generic name *Ampullacula*.

## 2. *Paraisotricha beckeri* Hsiung, 1930

(Pl. II, Fig. 27)

**Specific diagnosis.**—*Paraisotricha*: This protozoon resembles the preceding species in both structure and texture. It is ovoid, the anterior end being bluntly pointed and the posterior end rounded. The body is rigid, noncontractile, and almost twice as long as it is wide. It is widest at the middle. The body is covered with fine cilia, which are borne in grooves on tops of ridges. The sides of each groove appear to be two bright lines under the lower power of the microscope. These cilia-bearing ridges comprise only eleven spiral rows of striations. These striations run spirally forward from the aboral pole turning toward the left instead of toward the right as with the preceding species.

The cytostome is also a slanting slit on the ventral side of the anterior end, but it is situated more anterior than the preceding species. The esophagus is also ciliated. The cytophyge is a longitudinal slit at the posterior end of the body.

Both macronucleus and micronucleus resemble those of *P. colpoidea* and their locations in the body are also about the same as those of the preceding species.

The large concretion vacuole which contains six to fifteen refractive concretion granules occupies nearly the whole anterior end of the body. The large contractile vacuole has the same location as that of *P. colpoidea*. Reproduction is by transverse fission.

Measurements of 49 specimens yield the following results:

	Length	Width
Range .....	52 - 98 $\mu$	30 - 52 $\mu$
Mean .....	72.77 $\pm$ 0.98 $\mu$	41.57 $\pm$ 0.51 $\mu$
Standard deviation .....	10.28 $\mu$	5.32 $\mu$
Coefficient of variation ...	14.21 %	10.39 %

Habitat: cecum and colon.

Geographic distribution: U. S. A.

## 3. *Paraisotricha minuta* sp. nov.

(Pl. II, Figs. 28-32)

Synonym: *Paraisotricha colpoidea* Dogiel, 1929.

**Specific diagnosis.**—*Paraisotricha*: This protozoon resembles *P. colpoidea* in every respect, except that it is much smaller. The ciliary striations are only 20 in number and they run almost straight forward from their points of origin about the cytophyge. The concretion vacuole usually contains less than 10 small, very refractive concretion granules. The micronucleus is comparatively large and more refractive and is always situated at the end of the disc-shaped macronucleus. Reproduction is by transverse fission. Exconjugants have been observed. Specimens parasitized by *Sphaerita* have also been noted.



Measurements of 49 specimens yield the following results:

	Length	Width
Range .....	38 - 68 $\mu$	27 - 36 $\mu$
Mean .....	53.79 $\pm$ 0.57 $\mu$	31.95 $\pm$ 0.23 $\mu$
Standard deviation .....	6.03 $\mu$	2.39 $\mu$
Coefficient of variation ...	11.21 %	7.48 %

Habitat: cecum and colon.

Geographic distribution: Russia, U. S. A.

#### Family BLEPHAROCORIDAE Hsiung, 1929

Family diagnosis.—Trichostomata: Body elongated, generally flattened bilaterally; cytostome just ventral to anterior end, long esophagus provided with cilia throughout its length; tufts of cilia only at anterior and posterior ends; single contractile vacuole at posterior end.

Type-genus: *Blepharocorys* Bundle, 1895.

Hickson included the genus *Blepharocorys* in the family Chilifera Buetschli, 1887. Again Poche removed it to the family Colpodidae Claus, 1879. As referred above Cunha included it in his new family Paraisotrichidae. Since *Blepharocorys* does not have a concretion vacuole, which is common to the genus *Paraisotricha*, and the localized ciliary areas, the writer (1929) found it necessary to make a new family, *Blepharocoridae*, for the reception of the genera *Blepharocorys* Bundle and *Charon* Jameson.

#### Key to the Genera of *Blepharocoridae*

1. Anal cilia in a single bundle ..... *Blepharocorys*  
     Anal cilia in right and left bundles ..... *Charon*

#### Genus: *BLEPHAROCORYS* Bundle, 1895

Generic diagnosis.—*Blepharocoridae*: Oral vestibule present; with dorsal and ventral plates at anterior end, anal plate at posterior end; anal cilia in a single bundle.

Type-species: *Blepharocorys uncinata* (Fiorentini 1890) Bundle, 1895.

#### Key to Species of *Blepharocorys*

1. With anterior corkscrew-like process ..... *B. uncinata*  
     Without anterior process ..... 2
2. Anal cilia on dorsal side ..... *B. valvata*  
     Anal cilia on left side of anal plate ..... 3
3. Seen from the side macronucleus heart-shaped ..... *B. cardionucleata*  
     Seen from the side macronucleus not heart-shaped ..... 4
4. Esophagus bent at an angle more than 180° ..... *B. curvigula*  
     Esophagus not bent at an angle of 180° ..... 5
5. Dorsal plate toothed, body short ..... *B. jubata*  
     Dorsal plate not toothed, body elongated ..... *B. angusta*

1. *Blepharocorys uncinata* (Fiorentini, 1890) Bundle, 1895  
(Pl. II, Figs. 33-38)

Synonyms: *Diplodinium uncinatum* Fiorentini, 1890; *Blepharocorys equi* Schumacher, 1915.

Specific diagnosis.—*Blepharocorys*: This protozoon is elongated and irregular in shape, the dorsal side being slightly convex, the ventral side slightly concave, and the ends more or less rounded. The body is constant in form, rigid, noncontractile and about three times as long as it is thick. It is thickest in the region through the posterior margin of the ventral lip. It decreases slowly in thickness posteriad, but more rapidly anteriad. It is slightly flattened bilaterally.

The ectoplasm is a thin, refractive and homogeneous layer. The endoplasm appears to be slightly granular and contains small particles of food. The whole body is covered by a thin, firm and refractive cuticle. There are definite longitudinal striations on the cuticle in the living specimens.

From the dorsal part of the anterior end of this protozoon there projects a corkscrew-like anterior process. This process makes two turns and is rounded at its anterior extremity. It passes posteriad in an irregular path through the outer covering of the body and, gradually diminishing in thickness, passes along the dorsal side of the esophagus and finally ends just posterior to the end of the esophagus. It is clear, highly refractive, homogeneous in appearance, and of marked rigidity. Its path through the endoplasm can be traced as a bright line.

The anterior end contains a large vestibule, the lower wall of which is formed by a ventral lip. Its dorsal wall is formed by the frontal cap, which is covered both dorsally and ventrally with two dorsal plates. The left dorsal plate being much the larger of the two. In the anterior part the left dorsal plate extends laterally and ventrally around the anterior end of frontal cap and ends at a point just to the right and posterior to the corresponding end of the right dorsal plate. The main part of the plate arches away from the body slightly, curves ventrally around the left side of the body and extends along its whole edge over the left margin of the ventral lip, thus forming a left lateral wall of the vestibule. Posteriorly, however, its margin does not extend as far back as the posterior lateral edge of the vestibule; there is left in this region consequently an opening into the vestibule posterior to the left dorsal plate.

The right dorsal plate, like the left, extends laterally and ventrally around the end of the frontal cap. The remaining part simply arches away from the body laterally and bears little relation to the vestibule.

Along the ventral margin of the vestibule there is a row of adoral cilia. Situated on the anterior dorsal side of this ciliate there is a zone of cilia, the dorsal ciliary zone.

The cytostome is an irregular, poorly defined opening leading directly into the large funnel-shaped esophagus. It is situated at the left posterior end of the vestibule close to the ventral side of the body.

The esophagus lies in an oblique position with reference to the long axis of the body. It is directed dorso-posteriad. The posterior end bends sharply to the ventral side. Its lumen gradually narrows as the posterior end is approached and disappears at the posterior half of the body. The esophagus is ciliated along its dorsal wall with fine cilia, increasing in

length and continuous anteriorly with oral membranelles on the dorsal wall of the vestibule.

The macronucleus is situated in the anterior half of the body, dorsal and to the right of the esophagus. In general the macronucleus has a rather constant size, shape, and position in the animal. In side view it is heart-shaped, with the apex extending posteriad. On its dorsal surface near the right anterior side there is a small invagination in which the micronucleus partly lies. The macronucleus is distinctly granular. The micronucleus appears to be more or less homogeneous in structure and is ellipsoidal in shape.

The single contractile vacuole is situated in the posterior ventral part of the body in very close relation to the anal tube.

The cytopyge lies at the posterior end of the body slightly to the ventral side and to the right of the anal plate which is a semilunar posterior extension of the body cuticle. It is connected with an anal tube which extends into the body to the vicinity of the contractile vacuole.

The posterior end of the body around the cytopyge is covered with anal cilia. Reproduction is by transverse fission.

Measurements of 25 individuals yield the following figures:

	Length	Thickness
Range .....	55 - 74 $\mu$	22 - 30 $\mu$
Mean .....	65.24 $\pm$ 0.70 $\mu$	26.12 $\pm$ 0.29 $\mu$
Standard deviation .....	5.22 $\mu$	2.16 $\mu$
Coefficient of variation ...	8.00 %	8.26 %

The average width of some of the organisms measured is 22  $\mu$ . These figures show that they are slightly larger than those found by both Bundle and Schumacher.

Habitat: cecum and colon.

Geographic distribution: Italy, Germany, Brazil, S. Africa, U. S. A.

Schumacher (1915) made a new species *Blepharocorys equi* out of apparently the same protozoon, for he stated, "*Blepharocorys equi* may be distinguished from *Blepharocorys uncinata* (Fiorentini) by the shape of its frontal cap, which in *Blepharocorys equi* is covered by a right and left dorsal plate and by a zone of cilia on its dorsal surface. In *Blepharocorys uncinata* (Fiorentini), as described and figured by Bundle; there are no such plates present and the dorsal part of the frontal cap, i. e., the 'Sternkuppe' of Bundle, is not ciliated. Furthermore, there is present in *Blepharocorys equi* a ventral zone of adoral membranelles; this zone is not present in *Blepharocorys uncinata* (Fiorentini)." Although Bundle did not describe the dorsal plates, by looking over his figure one will surely notice that his "Querspalt" is the demarkation of the dorsal plates from the frontal cap. Furthermore, in Fiorentini's figure of *Diplodinium uncinatum* (= *Blepharocorys uncinata*) the letter "P" also shows the demarkation of the dorsal plates from the frontal cap. The misinterpretation on their part was undoubtedly due to lack of higher magnifications of their microscopes. That the "Sternkuppe" was ciliated was clearly stated in Bundle's description of the genus *Blepharocorys*, "Ueber dem Mund eine helmartige Stirnkuppe. Wimpern nur auf dieser, um Mund und After, sowie im Schlund; der übrige Körper nackt." But Fiorentini misinterpreted it as ciliary



corona of a second buccal orifice. As to the presence of the ventral zone of adoral membranelles Bundle described it as cilia around the mouth and Fiorentini showed it as a ciliary corona of the buccal orifice.

Therefore *Blepharocorys equi* Schumacher, 1915, can not be distinguished from *Blepharocorys uncinata* (Fiorentini, 1890), although Schumacher did give a better and more detailed description and excellent figures of the same protozoon, *Blepharocorys uncinata*. I am of the opinion that *Blepharocorys equi* Schumacher, 1915, should be made a synonym of *Blepharocorys uncinata* (Fiorentini, 1890).

As to the development of the anterior process the following observations were made. During the process of division there is not any sign of the extension of the posterior extremity of the anterior process to the posterior part of the body where the new individual is forming. Furthermore, there is not any indication of the presence of the anterior process on the newly formed esophagus. But after the division, the newly formed individual shows the presence of a bright line extending forward from the dorsal surface of the esophagus to the anterior end of the hook-like process of the dorsal plates. Further development shows that the anterior extremity of the anterior process breaks through the dorsal plates and gradually pushes out further.

## 2. *Blepharocorys valvata* (Fiorentini, 1890) Bundle, 1895 (Pl. II, Fig. 39)

Synonyms: *Entodinium valvatum* Fiorentini, 1890; *Blepharocorys microcorys* Gassovsky, 1919.

Specific diagnosis.—*Blepharocorys*: Seen from the side, this protozoon is more or less elliptical in shape. It is flattened bilaterally. It is about two and one-half times as long as it is thick. It is thickest at the middle of the body. Its general structure and texture are similar to the preceding species except that there are no cuticular striations.

The dorsal plate is small and beak-like. Its dorsal surface is strongly convex and its ventral surface, which forms the dorsal wall of the small vestibule, is slightly concave. There is a small ventral lip which guards the right side of the cytostome.

The small vestibule is not easy to perceive. The slightly curved esophagus, which is on the right side of the vestibule, directs upward and backward. It is ciliated along its dorsal wall.

There are three ciliary zones at the anterior end of the body. The first ciliary zone is at the dorsal surface of the base of the dorsal plate. A second zone is found on the ventral lip. The third zone is at the ventral side of the cytostome.

The macronucleus is more or less kidney-shaped. It is often found on the dorsal surface of the vestibule. A small subspherical micronucleus is usually found in the depression at the end of the macronucleus.

A contractile vacuole is located at the postero-ventral part of the body in the vicinity of the anal tube.

The cytophyge is located on the dorsal part of the body to the right side of the anal plate at the posterior end of the body. It is connected by



an anal tube. The anal cilia originate dorsal to the anus and also on the right side of the anal plate. Reproduction is by transverse fission.

Measurements of 12 specimens yield the following results:

Range .....	52 - 68 $\mu$	20 - 27 $\mu$
Mean .....	59.5 $\mu$	23.08 $\mu$

Habitat: cecum and colon.

Geographic distribution: Italy, Germany, S. Africa, U. S. A.

According to Fiorentini's figure of *Entodinium valvatum* (= *Blepharocorys valvata*) it resembles *Blepharocorys microcorys* closely. They show the same dorsal location of the anus and the anal cilia. But according to Bundle's figure of *Blepharocorys valvata*, it is an entirely different animal from either *E. valvatum* figured by Fiorentini or *B. microcorys* figured by Gassovsky. The shape of the body and ciliation on the dorsal plate are entirely erroneously described. If it were not for its curved esophagus and the dorsal location of anus and anal cilia it could not be reconciled with *B. valvata*.

3. *Blepharocorys jubata* Bundle, 1895  
(Pl. II, Fig. 40)

Specific diagnosis.—*Blepharocorys*: Its general shape and its texture are more or less similar to the preceding species. The body is more than twice as long as it is thick. It is thickest in the region just back of the base of the dorsal plate. It decreases slowly in thickness posteriad, but quite rapidly anteriorly.

There is an incomplete vestibule at the anterior end. Its dorsal wall is formed by the dorsal plate, which is round on the right side and toothed on the left side. Its dorsal surface is more or less convex and its ventral surface is concave. The ventral and right lateral walls are formed by the concave, semilunar ventral lip.

The cytostome at the left side is irregular and is guarded on the dorsal side by the two teeth of the dorsal plate. The cytostome is just a slanting slit on the right side between the dorsal plate and the ventral lip. The vestibule leads directly to the esophagus which is situated mainly on the left side. The esophagus goes backward and upward and then again turns backward. It is ciliated on its dorsal wall and the ciliation extends to the dorsal wall of the vestibule.

The dorsal surface of the dorsal plate is ciliated at the base. The base of the ventral lip is also ciliated. There is another bundle of cilia on the left side at the base of the ventral lip.

The ctyopyge lies at the posterior end of the body slightly to the dorsal side and at the left side of the anal plate. It is connected by an anal tube which extends to the vicinity of the contractile vacuole. Anal cilia are found at the posterior end of the body to the left of the anal plate.

The macronucleus is more or less ovoid. It is granular and is situated at the anterior end to the right of the esophagus. A small subspherical micronucleus is usually found in the depression of the macronucleus.

A contractile vacuole is usually found at the posterior end at the vicinity of the anus. Reproduction is by transverse fission.

Measurements of 25 individuals yield the following results:

	Length	Thickness
Range .....	33 - 60 $\mu$	17 - 23 $\mu$
Mean .....	47.12 $\pm$ 0.88 $\mu$	20.16 $\pm$ 0.26 $\mu$
Standard deviation .....	6.62 $\mu$	1.97 $\mu$
Coefficient of variation ...	14.04 %	9.77 %

I have never found any concretion vacuole in *Blepharocorys jubata* as described by Bundle.

Habitat: cecum and colon.

Geographic distribution: Germany, Brazil, U. S. A.

#### 4. *Blepharocorys curvigula* Gassovsky, 1919

(Pl. II, Fig. 41)

Specific diagnosis.—*Blepharocorys*: In general shape and texture this protozoon resembles the preceding two species. Its body is about three times as long as it is thick. It is thickest in the region through the middle of the macronucleus. It decreases slowly in thickness posteriad, but more rapidly anteriad. It is flattened bilaterally.

The anterior end contains a vestibule, the dorsal and left walls of which are formed by the dorsal plate. The ventral lip forms part of its right wall. The cytostome is irregular and leads directly to a long esophagus, which is to the left of the vestibule and which directs backward and upward and finally bends in a smooth curve of 180°. It is ciliated along its dorsal wall and the ciliation extends into the vestibule.

The dorsal plate is a more or less rhomboid plate with its dorsal and left sides convex and the right side concave. The antero-ventral angle is pointed and antero-dorsal angle is rounded. There are three ciliary zones at the anterior end of the body. There is a bundle of cilia at its dorsal base. The base of the ventral lip also has a row of cilia. The third bundle of cilia is situated at the ventral side of the body near the base of the ventral lip.

The cytopyge lies at the posterior end of the body slightly to the dorsal side and at the left side of the anal plate. It is connected with an anal tube which extends to the vicinity of the large contractile vacuole. Anal cilia are found to the left of the anal plate.

The macronucleus is more or less ovoid. It is granular and situated at the anterior end dorsal to the vestibule and to the right side of the esophagus. A small subspherical micronucleus is situated in the depression of the macronucleus.

A large contractile vacuole is usually found at the posterior end of the body in the vicinity of the anal tube. Reproduction is by transverse fission.

Measurements of 25 individuals yield the following results:

	Length	Thickness
Range .....	76 - 99 $\mu$	29 - 37 $\mu$
Mean .....	87.28 $\pm$ 0.75 $\mu$	32.68 $\pm$ 0.29 $\mu$
Standard deviation .....	5.64 $\mu$	2.20 $\mu$
Coefficient of variation ...	6.26 %	6.73 %

Habitat: colon.

Geographic distribution: Russia, U. S. A.

### 5. *Blepharocorys angusta* Gassovsky, 1919

(Pl. II, Fig. 42)

Specific diagnosis.—*Blepharocorys*: In general shape and texture this protozoon resembles the preceding three species except that it is elongated. It is more than three times as long as it is thick. It is thickest in the region just posterior to the base of the frontal cap. The dorsal plate occupies the dorsal side and the entire left side of the anterior end.

Seen from the left side the dorsal plate is more or less a rhomboid plate with the ventral border slightly concave. Its antero-dorsal corner is rounded and its antero-ventral corner is pointed. The triangular ventral lip lies on the right side and a little to the ventral side.

There are three ciliary zones at the anterior end of the body. It is ciliated on the left side of the base of the dorsal plate. The base of the ventral lip also possesses a row of cilia. The third bundle of cilia is found on the antero-ventral edge of the body.

The cytostome lies between the dorsal plate and the ventral lip. It leads directly to the long, straight esophagus which is ciliated. The esophagus lies close to the left side of the body.

The cytopyge lies at the posterior end of the body slightly to the dorsal side and at the left of the anal plate. It is connected with a short anal tube. Anal cilia are found to the left of the anal plate.

The macronucleus is irregular. It is granular and is situated at the anterior end just ventral and lateral to the right side of the esophagus. A subspherical micronucleus is located in the depression of the macronucleus.

The contractile vacuole is located at the posterior end a little to the ventral side in the vicinity of the anal tube. Reproduction is by transverse fission.

Measurements of 25 individuals yield the following figures:

	Length	Thickness
Range .....	58 - 78 $\mu$	20 - 25 $\mu$
Mean .....	70.12 $\pm$ 0.76 $\mu$	22.52 $\pm$ 0.20 $\mu$
Standard deviation .....	5.68 $\mu$	1.50 $\mu$
Coefficient of variation ...	8.10 %	6.66 %

Habitat: colon.

Geographic distribution: Russia, U. S. A.

### 6. *Blepharocorys cardio-nucleata* sp. nov.

(Pl. II, Fig. 43)

Specific diagnosis.—*Blepharocorys*: In general shape and texture this protozoon resembles *B. curvigula* closely. Its body is about two and one-half times as long as it is thick. It is thickest at the middle of the body. It is flattened bilaterally.

The anterior end contains a small vestibule which leads to the slightly curved esophagus. This esophagus directs upward and backward and turns downward at the posterior third of the body. It is also ciliated along its dorsal wall and the ciliation extends into the vestibule.

The dorsal plate also resembles that of *B. curvigula*. The ventral lip is situated entirely on the right side of the cytostome. The three ciliary zones are also similar to those of *B. curvigula*.

The cytophyge lies at the posterior end of the body slightly to the dorsal side and at the left side of the anal plate. It is connected with an anal tube. Anal cilia are also found on the left side of the anal plate.

The macronucleus is heart-shaped with an anterior base and a posterior apex. In this respect it resembles *B. uncinata* but in the same respect it is sharply separated from the other species of this genus. It is slightly granular and is situated at the anterior end and to the right side of the esophagus. A small spherical micronucleus is situated in the depression at the base of the macronucleus.

A contractile vacuole is found at the posterior end of the body a little to the ventral side at the vicinity of the anal tube. Reproduction is by transverse fission.

Measurements of 25 specimens yield the following results:

	Length	Thickness
Range .....	48 - 62 $\mu$	17 - 23 $\mu$
Mean .....	55.12 $\pm$ 0.55 $\mu$	20.24 $\pm$ 0.51 $\mu$
Standard deviation .....	4.10 $\mu$	3.87 $\mu$
Coefficient of variation ...	7.43 %	19.12 %

Habitat: colon.

Geographic distribution: U. S. A.

Genus: *CHARON* Jameson, 1925

Generic diagnosis.—Blepharocoridae: Body not flattened bilaterally; anterior knob present; anal cilia in right and left bundles.

Type-species: *Charon ventriculi* Jameson, 1925.

#### Key to Species of *Charon*

1. Found in large intestine of the horse ..... *C. equi*  
Found in stomachs of ruminants ..... *C. ventriculi*

#### *Charon equi* Hsiung, 1930

(Pl. II, Fig. 44)

Specific diagnosis.—*Charon*: Seen from dorsal side, the shape of this protozoon is lancet; the right side is slightly convex, the left side is more so. The anterior end is bluntly pointed while the posterior end is nearly rounded. It is about three times as long as it is wide. It decreases slowly in width posteriad but more rapidly anteriad thus forming an anterior projecting knob. Both dorsal and ventral surfaces are slightly convex.

The ectoplasm is homogeneous. The endoplasm is finely granular and contains small particles of food.



The cytostome, which occupies nearly the whole ventral side of the anterior knob, is more or less triangular in form. It leads directly into a prominent esophagus which is ciliated and which extends straight down to the middle third of the body. There are three ciliary zones at the anterior end. The right zone is situated on the right margin while the left zone is situated on the left margin of the cytostome. The small dorsal zone originates from the dorsal side of the knob.

The cytopyge is sometimes visible as a dark line situated between two bundles of cilia at the posterior end. A single contractile vacuole can usually be seen in the posterior part of the body.

There is a large elongated ellipsoid macronucleus which varies in its position in the body. A comparatively large ovoid micronucleus is usually found some distance from the macronucleus.

The measurements of 49 specimens yield the following results:

	Length	Width
Range .....	30 - 48 $\mu$	10 - 14 $\mu$
Mean .....	39.5 $\pm$ 0.305 $\mu$	11.8 $\pm$ 0.004 $\mu$
Standard deviation .....	3.17 $\mu$	0.04 $\mu$
Coefficient of variation ...	8.0 %	0.34 %

Habitat: colon.

Geographic distribution: U. S. A.

Order: **Heterotrichida** Delage and Hérourard, 1896

Family: **BURSARIIDAE** Kent, 1880

Genus: *BALANTIDIUM* Claparède and Lachmann, 1858

**Balantidium coli** (Malmsten, 1857) Stein, 1863

Specific diagnosis.—Cunha (1917) observed *Balantidium coli* in the material from the large intestine of the horse. The following is the translation of his original description of the parasite found in the horse:

“This ciliate presents a body of ovoid form with the anterior extremity narrower. The body is uniformly covered with fine cilia, arranged in longitudinal lines, which give to the ciliate a striated aspect. At the anterior extremity is observed the very short peristome in the form of a triangular cleft, at the left side of which is an adoral zone made up of cilia thicker than those which cover the body.

“This ciliate presents two contractile vacuoles, one located in the middle part and the other in the posterior part of the body. In the stained preparations is observed the reniform macronucleus, in the depression of which exists a micronucleus.—The dimensions of the parasite are: length 40 to 60  $\mu$ , width 35 to 40  $\mu$ .”

The writer failed to find any ciliate which resembles *Balantidium* from the intestinal materials collected from more than forty horses.

Geographic distribution: Brazil.

Order: **Oligotrichida** Wenyon, 1926

Order diagnosis.—Body mostly unciliated except for certain special zones.

Family: **CYCLOPOSTHIIDAE** Poche, 1913

Family diagnosis.—Generally, body rigid, flattened bilaterally; adoral zone of membranelles; possessing either caudalia or arches of membranelles.

Type-genus: *Cycloposthium* Bundle, 1895.

*Key to Genera of Cycloposthiidae*

1. Body more or less cylindrical ..... *Spirodinium*  
    Body flattened bilaterally ..... 2
2. Body skeleton present; ciliophore retractile. .... 3  
    Body skeleton absent; no retractile ciliophore ..... 4
3. Only two posterior caudalia or arches ..... *Cycloposthium*  
    Two posterior caudalia and an anterior caudalium ..... *Tripalmaria*
4. Three arches of membranelles ..... 5  
    Four arches of membranelles ..... 6
5. Body galeate ..... *Triadinium*  
    Body elongated ..... *Ditoxum*
6. Body length more than 200  $\mu$ ; both ends of macronucleus  
    curved ..... *Cochliatorum*  
    Body length less than 180  $\mu$ ; only anterior end of macronucleus  
    curved ..... *Tetratorum*

Hickson (1903) referred the genus *Cycloposthium* to the family *Ophryoscolecina* Stein, 1858. Poche (1913) made for it a new family, *Cycloposthiidae*, to which the genus *Didesmis* was also assigned. Cunha (1919) removed the genus *Didesmis* to the family *Buetschliidae*. The writer includes in the family *Cycloposthiidae* the following genera: *Cycloposthium* Bundle, 1895; *Spirodinium* Fiorentini, 1890; *Triadinium* Fiorentini, 1890; *Tetratorum* Gassovsky, 1919; *Tripalmaria* Gassovsky, 1919; *Cochliatorum* Gassovsky, 1919 and *Ditoxum* Gassovsky, 1919.

Genus: **CYCLOPOSTHIUM** Bundle, 1895

Generic diagnosis.—*Cycloposthiidae*: Body covered with skeleton; anterior retractile peristome with adoral membranelles; two posterior caudalia or arches of membranelles, a dorsal and a ventral; elongated macronucleus; contractile vacuoles in longitudinal row.

Type-species: *Cycloposthium biplamatum* (Fiorentini, 1890) Bundle, 1895.

*Key to the Species of Cycloposthium*

1. Posterior arches of membranelles nonretractile ..... *C. ishikawai*  
    Posterior caudalia retractile ..... 2

2. Presences of ventral dentiform projection; anterior end of macronucleus not hooked. .... 3  
Absence of ventral dentiform projection; anterior end of macronucleus hooked ..... 5
3. Cuticle corrugated ..... *C. corrugatum*  
Cuticle not corrugated ..... 4
4. Tooth strongly developed, more flattened; greater length of body (135-227  $\mu$ ) ..... *C. dentiferum* f. *latidens*  
Tooth not strongly developed, less flattened; body length shorter (122-180  $\mu$ ) ..... *C. dentiferum*
5. Absence of both longitudinal groove and linear skeleton on the left side of the body ..... *C. piscicauda*  
Presence of both longitudinal groove and linear skeleton on the left side of the body. .... 6
6. Presence of shield on the left side of the body ..... *C. scutigerum*  
Absences of shield on the left side of the body ..... 7
7. Body large, usually over 150  $\mu$  in length; contractile vacuole usually more than four in number..... *C. edentatum*  
Body small, less than 150  $\mu$  in length; contractile vacuole usually four in number ..... 8
8. Heavy skeleton; micronucleus located near to the anterior end of the macronucleus ..... *C. affinae*  
Light skeleton; micronucleus located at the middle of the macronucleus ..... *C. bipalmatum*

1. *Cycloposthium bipalmatum* (Fiorentini, 1890) Bundle, 1895  
(Pl. III, Figs. 45-46)

Synonym: *Entodinium bipalmatum* Fiorentini, 1890.

Specific diagnosis.—*Cycloposthium*: Body form is constant and is more or less rectangular. It is slightly compressed laterally. The anterior end is truncated while the posterior end is tapering and finally forms a tail-like structure. Posterior end of the dorsal surface slopes down while the ventral surface is straight but grooved. The dorsal and ventral caudalia are asymmetrically situated just anterior to the tail. The dorsal caudalium is more posterior than the ventral one. The presence of the light skeleton, which never extends to the tail and the caudalia, makes the body fairly rigid. A slight groove which runs parallel to the long axis of the body is present on the left surface near to the dorsal side. A linear skeleton can be seen just ventral to the groove.

The peristome is located on the ciliophore at the anterior end of the body. It is surrounded by two rows of membranelles and the collar. The ctyopyge is situated behind the base of the ventral caudalium and it is connected by an anal tube.

The elongated macronucleus lies just under the dorsal surface and runs parallel with the long axis of the body. Its anterior end forms a short hook toward the right side. The small ellipsoidal micronucleus lies in a depression on the dorsal side about the middle of the macronucleus.

There are four contractile vacuoles, some of which lie just above the linear skeleton. Reproduction is by transverse fission.

Measurements of 25 specimens yield the following results:

	Length	Thickness
Range .....	80 - 127 $\mu$	35 - 57 $\mu$
Mean .....	101.64 $\pm$ 1.75 $\mu$	41.96 $\pm$ 0.81 $\mu$
Standard deviation .....	13.07 $\mu$	6.10 $\mu$
Coefficient of variation ...	12.85 %	14.53 %

Conjugating forms have also been observed. This organism apparently feeds on bacteria and plant fibers, but once a specimen containing a nematode larva has also been observed.

Habitat: both cecum and colon.

Geographic distribution: cosmopolitan.

## 2. *Cycloposthium dentiferum* Gassovsky, 1919, partim, Strelkow, 1928 (Pl. III, Fig. 47)

Specific diagnosis.—*Cycloposthium*: Body form is constant and irregularly rectangular. It is slightly compressed laterally. Its anterior end is truncated while its posterior end tapers sharply to form a tail-like structure. Its dorsal side is gently curved while its ventral side is strongly convex to form a dental process which points forward. The dorsal and ventral caudalia are asymmetrically placed just anterior to the tail like the preceding species. The entire surface, except the caudalia and the tail, is covered with skeleton, which gives the body its rigidity. The longitudinal groove is present on the left side of the body but the linear skeleton is quite indistinct.

The peristome is located on the ciliophore at the anterior end of the body. It is surrounded by two rows of membranelles and the collar. The cytopye is situated behind the base of the ventral caudalium and it is connected by an anal tube.

The elongated macronucleus lies just under the dorsal surface and runs parallel with the long axis of the body. Its anterior end slightly tapers while its posterior end is slightly enlarged and obliquely truncated running parallel with the base of the dorsal caudalium. The subspherical micronucleus lies in a depression on the left side about the middle of the macronucleus.

There are four to six contractile vacuoles which lie beneath the left surface in direct opposition to the macronucleus. Reproduction is by transverse fission. Exconjugants have also been observed. The organism is usually engorged with plant fibers.

Measurements of 25 specimens yield the following results:

	Length	Thickness
Range .....	140 - 222 $\mu$	80 - 110 $\mu$
Mean .....	178.48 $\mu$	93.92 $\mu$

Habitat: cecum and colon.

Geographic distribution: Russia, U. S. A.



### 3. *Cycloposthium dentiferum* forma *latidens* Strelkow, 1929

Specific diagnosis.—*Cycloposthium*: Except for the following differences, this form resembles *C. dentiferum* in every respect. It differs from the typical *C. dentiferum* by the great development of the ventral tooth-like process and by the greater length of the body. Its tooth-like process is more strongly flattened than that of the typical *C. dentiferum*.

Measurements: Body length 135-227  $\mu$  (mean 185  $\mu$ ), thickness 56-100  $\mu$  (mean 81  $\mu$ ).

The writer has not observed this form in the American horses.

Habitat: cecum.

Geographic distribution: Russia.

### 4. *Cycloposthium ishikawai* Gassovsky, 1919

(Pl. III, Fig. 48)

Specific diagnosis.—*Cycloposthium*: "Body obtusely truncated anteriorly, gradually narrowing posteriorly. Anterior part of dorsal side dilated in the form of wings. Both ends of macronucleus thickened and bent towards ventral margin. Membranellae of posterior end composing two caudal arches. Measurements: 230-280 x 110-130  $\mu$ . Cecum of Japanese horses."

This organism has not been observed in the American horses.

### 5. *Cycloposthium edentatum* Strelkow, 1928

(Pl. III, Fig. 49)

Specific diagnosis.—*Cycloposthium*: Body form is constant and more or less rectangular. It is slightly compressed laterally. Its anterior end is truncated while its posterior end tapers to form a curved tail. The dorsal side is slightly convex while the ventral side is flat. The dorsal and ventral caudalia are asymmetrically placed just anterior to the tail. The cuticular skeleton extends to part of the tail. The skeleton on the left side of the body is interrupted by a longitudinal groove which is just dorsal to the longitudinal skeleton inside.

The peristome with its adoral membranellae is located at the anterior end. The cytotpyge is situated behind the base of the ventral caudalium and is connected by an anal tube.

The elongated macronucleus is situated under the dorsal surface parallel with the long axis of the body. Its anterior end is curved toward the right side to form a short hook. Its posterior extremity is slightly enlarged and obliquely truncated running parallel to the base of the dorsal caudalium. The ellipsoidal micronucleus is situated in a depression on the left side at the anterior third of the macronucleus.

There are 6 to 7 contractile vacuoles which lie ventral to the longitudinal skeleton. Reproduction is by transverse fission.

Measurements of 25 specimens yield the following results:

	Length	Thickness
Range .....	146 - 230 $\mu$	68 - 93 $\mu$
Mean .....	190.04 $\mu$	80.22 $\mu$

Its food materials are consisted of mainly plant fibers.

Habitat: cecum and colon.

Geographic distribution: Russia, U. S. A.

## 6. *Cycloposthium piscicauda* Strelkow, 1928

(Pl. III, Fig. 50)

Specific diagnosis.—*Cycloposthium*: Body shape is constant and has a rectangular form. It is compressed laterally. Its ventral side is more flat than the dorsal side. The anterior extremity is truncated, while the posterior end forms a tail resembling that of a fish. The posterior end of the cuticular skeleton extends to the tail.

The peristome is anterior and the two caudalia are asymmetrically arranged at the posterior end as with other species. The anal opening is at the usual place, the base of the ventral caudalium.

The elongated macronucleus is found on the dorsal side a little to the left. It is slightly curved toward ventral and a little to the right. Its anterior end is curved toward the right side in the form of a long hook. The posterior extremity of the macronucleus is slightly enlarged and is obliquely truncated in the direction parallel to the base of the dorsal caudalium. The ellipsoidal micronucleus is situated near to the region where the macronucleus is curved to form the hook.

The contractile vacuoles are four to five in number and are situated on the left side of the body near to the macronucleus.

The length of the body varies from 125 to 190  $\mu$  (mean 152  $\mu$ ); the thickness of the body is from 44 to 80  $\mu$  (mean 65  $\mu$ ).

Strelkow has seen this form only once in association with *C. bipalmatum*.

Geographic distribution: Russia.

## 7. *Cycloposthium scutigerum* Strelkow, 1928

(Pl. III, Fig. 51)

Specific diagnosis.—*Cycloposthium*: Body form is constant and more or less rectangular. It is also slightly compressed laterally. Its anterior end is truncated while the posterior end tapers to form a curved tail. Its dorsal side is slightly convex while its ventral side is flat with a slight groove in the middle. On the left side of the body the skeleton is interrupted by a deep longitudinal groove near to the dorsal side. Another deep longitudinal groove at the middle of the body separates a skeletal plate from the rest of the body except for a narrow region between these two grooves. Under this raised plate there are two longitudinal skeletal lines which are really the skeletal walls of these two grooves. The two caudalia are asymmetrically placed at the posterior end.

The peristome and the adoral membranelles are located at the anterior end of the body. The cytopyge lies behind the base of the ventral caudalium and it is connected by an anal tube.

The elongated macronucleus lies just under the dorsal surface and runs parallel with the long axis of the body. Its anterior end is curved toward the right to form a hook. Its posterior end is curved slightly toward the

ventral side. The ellipsoidal micronucleus is situated in a depression on the left side at the anterior third of the macronucleus.

There are 5 to 6 contractile vacuoles which are situated between those two longitudinal skeletal lines. Reproduction is by transverse fission.

Measurements of 25 specimens yield the following results:

	Length	Thickness
Range .....	132-210 $\mu$	63-90 $\mu$
Mean .....	155.36 $\mu$	74.36 $\mu$

Habitat: cecum and colon.

Geographic distribution: Russia and U. S. A.

### 8. *Cycloposthium affinae* Strelkow, 1928

(Pl. III, Fig. 52 and Pl. IV, Figs. 53-55)

Specific diagnosis.—*Cycloposthium*: Body shape is constant and is more or less rectangular. It is slightly compressed laterally. The anterior end is obtusely truncated while the posterior end is tapering and finally forms a tail-like structure which resembles more or less that of *C. piscicauda*. The dorsal and ventral caudalia are asymmetrically placed just anterior to the tail like the other species. The caudalia are rather large with respect to the small body size. The rigidity of the body is due to the presence of an external skeleton which is especially thick on the dorsal surface and at the anterior fifth of the ventral surface. The thick skeleton of both lateral surfaces is limited to only that area bounded posteriad by a line connecting the posterior ends of the dorsal and ventral skeletons. This thick skeleton is interrupted on the left surface by a slight groove which runs parallel to the long axis of the body in the vicinity of the macronucleus. A linear skeleton can be seen just ventral to the groove. The rest of the body, with the exceptions of the tail and the caudalia which are destitute of skeleton, is supplied with only a thin skeleton.

The peristome is located at the anterior end near to the dorsal side of the body. It is surrounded by two rows of membranelles. The anal tube which originates from the endoplasm of the ventral side opens by an orifice behind the base of the ventral caudalium.

The elongated macronucleus lies just under the dorsal skeleton and runs parallel with the long axis of the body. It is slightly curved toward the right side at the posterior end. At the anterior end the macronucleus forms a short hook toward the right side. An ellipsoidal micronucleus is situated in a depression on the left surface of the macronucleus just below the curvature where the hook originates.

The four contractile vacuoles are situated on the left side of the body just ventral to the linear skeleton. They are always clearly visible. Reproduction is by transverse fission. Different stages of the exconjugants have also been observed.

The measurements of 49 specimens yield the following results:

	Length	Thickness
Range .....	92-141 $\mu$	45-58 $\mu$
Mean .....	120.2 $\pm$ 1.10 $\mu$	51.8 $\pm$ 0.25 $\mu$
Standard deviation .....	11.5 $\mu$	2.5 $\mu$
Coefficient of variation ...	9.56 %	4.82 %

Habitat: cecum and colon.

Geographic distribution: Russia, U. S. A.

### 9. *Cycloposthium corrugatum* sp. nov.

(Pl. IV, Fig. 56)

Specific diagnosis.—*Cycloposthium*: In body form this protozoon resembles more closely that of *C. dentiferum* than that of any other species of the genus. It is irregularly rectangular. Its anterior end is truncated, while its posterior end tapers to form the curved tail. Both its dorsal and ventral surfaces are slightly curved while the lateral surfaces are strongly convex. The anterior end of the ventral side also forms a dental process which is slightly developed. The entire surface, with the exception of the tail and the two caudalia, is covered with skeleton which is longitudinally corrugated with 9 to 10 lines on each lateral surface. The longitudinal groove is present on the left surface near to the dorsal side but the linear skeleton is quite indistinct.

The peristome with its adoral membranelles is located at the anterior end. The two caudalia are asymmetrically placed just anterior to the tail. The cytopyge lies behind the base of the ventral caudalium and it is connected by an anal tube.

The elongated macronucleus lies just under the dorsal surface, a little toward the right side and runs parallel with the long axis of the body. It is curved in conformity with the curvature of the dorsal side. Its anterior end is rounded while its posterior end is obliquely truncated running parallel to the base of the dorsal caudalium. The ellipsoidal micronucleus lies in a depression on the left side just below the middle of the macronucleus.

There are 4 to 5 contractile vacuoles which lie under the left surface of the body in direct opposition to the macronucleus. Reproduction is by transverse fission.

Measurements of 25 specimens yield the following results:

	Length	Thickness
Range .....	135 - 195 $\mu$	70 - 112 $\mu$
Mean .....	164.68 $\mu$	90.72 $\mu$

Habitat: cecum and colon.

Geographic distribution: U. S. A.

### Genus: *SPIRODINIUM* Fiorentini, 1890

Generic diagnosis.—*Cycloposthiidae*: Body elongated, elastic, more or less spindle-shaped; with an adoral zone of membranelles; zone of membranelles near anterior end making at least one complete spiral; a posterior zone of membranelles making only a half-spiral.

Type-species: *Spirodinium equi* Fiorentini, 1890; only species of the genus.



**Spirodinium equi Fiorentini, 1890**

(Pl. IV, Figs. 57-58)

Specific diagnosis.—*Spirodinium*: It is about two and one-half times as long as it is wide. It is widest at the middle. The ectoplasm is a thin homogeneous layer. The endoplasm is coarsely granular and containing refractile spherical bodies, plant fibers as well as other food particles. The whole body is covered by a finely punctate cuticle. There are longitudinal striations on its dorsal surface under the cuticle. The anterior zone of membranelles starting from the ventral side makes a complete spiral, extending over a little to the left side. The posterior arch of membranelles is situated on the dorsal side, a little to the left.

The peristome is at the anterior extremity on the ventral side. It is surrounded entirely by the adoral membranelles. The cytopyge is located at the posterior end on the ventral side under the caudal sheath.

The macronucleus is elongated with both ends rounded. It is distinctly granular. It is situated under the dorsal surface between the two zones of membranelles. A large ellipsoidal micronucleus is located on the right side and at the posterior half of the macronucleus.

A large contractile vacuole is usually found under the right surface just back of the anterior membranelles. Reproduction is by transverse fission.

Measurements of 49 specimens yield the following results:

	Length	Width
Range .....	77 - 180 $\mu$	30 - 74 $\mu$
Mean .....	127.69 $\mu$	49.71 $\mu$

Habitat: colon.

Geographic distribution: Italy, S. Africa, U. S. A.

**Genus: *TRIADINIUM* Fiorentini, 1890**

Generic diagnosis.—Cycloposthiidae: Body somewhat helmet shaped and flattened bilaterally; a wide peristome surrounded with adoral membranelles; two other arches of membranelles.

Type-species: *Triadinium caudatum* Fiorentini, 1890

*Key to Species of Triadinium*

1. Tail absent; macronucleus elongated ..... *T. galea*
- Tail present; macronucleus not elongated ..... 2
2. Size large, more than 50  $\mu$  in length; macronucleus twisted as an  
interrogative mark ..... *T. caudatum*
- Size small, less than 50  $\mu$  in length; macronucleus rounded. . *T. minimum*

**1. *Triadinium caudatum* Fiorentini, 1890**

(Pl. IV, Figs. 59-60)

Specific diagnosis.—*Triadinium*: Body is constant in form, rigid and noncontractile.

The ectoplasm is a thin and homogeneous layer. The endoplasm is granular and contains great masses of food, especially fungus spores. The whole body is covered by a thin, firm and refractive cuticle. At the posterior end it forms a knob where the long, slender tail is attached. This tail is not a tuft of long cilia as referred to by Fiorentini.

At the anterior end of the body there is the short anterior arch of membranelles. The dorsal arch of membranelles is located at the posterior half of the dorsal surface of the body. The wide peristome, which is situated at the middle of the ventral surface of the body, is surrounded by a thick row of fine adoral membranelles. The funnel shaped esophagus is directed antero-dorsally. The cytopyge lies at the posterior end of the body dorsal to the caudal knob. It is connected by a distinct anal canal which is directed antero-dorsally just under the dorsal arch.

The macronucleus, which is twisted like an interrogative mark, is situated at the antero-ventral part of the body. The heavy part is usually directed ventrally, while the tail points dorsally. It is distinctly granular. A small subspherical micronucleus is usually found on the tail of the macronucleus. A single contractile vacuole is usually found anterior to the curvature of the macronucleus. Reproduction is by transverse fission.

Measurements of 49 specimens yield the following results:

	Length	Thickness
Range .....	50 - 105 $\mu$	36 - 85 $\mu$
Mean .....	72.88 $\mu$	55.16 $\mu$

Habitat: colon.

Geographic distribution: Italy, S. Africa, U. S. A.

## 2. *Triadinium galea* Gassovsky, 1919 (Pl. IV, Figs. 61-64)

Specific diagnosis.—*Triadinium*: Body is constant in form, rigid and noncontractile.

The ectoplasm is a thin and homogenous layer. The endoplasm is granular and contains food particles. The whole body is also covered by a thin, firm and refractive cuticle. The peristome is anterior. It is on the right side of a flattened conical cuticular projection. It is surrounded by an incomplete circular row of oral membranelles. A large funnel-shaped esophagus is present. On the right side of the oral opening there is another cuticular projection which separates it from the dorsal arch of membranelles. The other arch of membranelles is posterior. The cytopyge escaped detection.

The macronucleus is situated near to the left surface and along the long axis of the body. It is distinctly granular. Both ends of the macronucleus are bent toward the dorsal surface. The anterior end is the larger of the two. At the tip of the posterior end there is the small ellipsoidal micronucleus.

At the anterior curvature of the macronucleus there is usually a contractile vacuole. A second contractile vacuole can usually be found dorsal to the posterior arch of membranelles and near to the posterior end of the macronucleus.

Measurements of 49 specimens yield the following results:

	Length	Thickness
Range .....	53 - 88 $\mu$	50 - 70 $\mu$
Mean .....	68.75 $\pm$ 0.49 $\mu$	57.36 $\pm$ 0.43 $\mu$
Standard deviation .....	5.16 $\mu$	4.51 $\mu$
Coefficient of variation ...	7.50 %	8.45 %

Reproduction seems to be by longitudinal fission. The micronucleus accompanies the macronucleus to lie at the posterior end of the body. There is a longitudinal groove on either side of the region of the cytostome. The grooves are wider at the anterior end and narrower at the posterior end. Both nuclei have been observed in division. At this time a new paristome forms while both dorsal and posterior arches are still single. Further processes of division have not been observed.

Habitat: colon.

Geographic distribution: Russia, U. S. A.

### 3. *Triadinium minimum* Gassovsky, 1919

(Pl. IV, Fig. 65)

Specific diagnosis.—*Triadinium*: Body resembles *T. caudatum* more closely than *T. galea*.

The ectoplasm is a thin homogeneous layer. The endoplasm is finely granular. A thin cuticle is also present. At the posterior end it prolongs into a caudal process at the end of which the slender tail is attached. At the left side of the peristome there is another cuticular projection which forms a spine.

At the anterior end of the body is the short arch of fine membranelles. The dorsal arch of membranelles is also situated at the posterior half of the body. The wide peristome is situated on the ventral side at the posterior half of the body. It is surrounded by fine adoral membranelles. Back of the peristome there seems to be a vestibule to which a long curved esophagus is connected. Both esophagus and vestibule are ciliated. The cytopyge is situated at the posterior end dorsal to the caudal process.

The macronucleus is ellipsoidal in shape and is usually situated at the center of the body. A small subspherical micronucleus is usually found at one end of the macronucleus.

A contractile vacuole is located at the posterior end of the body near to the cytopyge. Dividing forms have not been encountered.

Measurements of 36 specimens yield the following results:

	Length	Thickness
Range .....	32 - 50 $\mu$	31 - 42 $\mu$
Mean .....	42.86 $\pm$ 0.55 $\mu$	36.08 $\pm$ 0.37 $\mu$
Standard deviation .....	4.98 $\mu$	3.33 $\mu$
Coefficient of variation ...	11.61 %	9.22 %

Habitat: colon.

Geographic distribution: Russia, U. S. A.

Genus: *TETRATOXUM* Gassovsky, 1919

Generic diagnosis.—Cycloposthiidae: Body slightly flattened bilaterally; two anterior arches of membranelles and two posterior arches of membranelles.

Type-species: *Tetratoxum unifasciculatum* (Fiorentini, 1890) Gassovsky, 1919.

*Key to Species of Tetratoxum*

1. Body small, less than 100  $\mu$  in length; without longitudinal cuticular ridges ..... *T. parvum*  
 Body large, generally over 100  $\mu$  in length; with longitudinal cuticular ridges ..... 2
2. Raised cuticular flap on the right anterior end of the body ..... *T. excavatum*  
 No raised cuticular flap on the right anterior end of the body ..... *T. unifasciculatum*

1. *Tetratoxum unifasciculatum* (Fiorentini, 1890) Gassovsky, 1919  
 (Pl. V, Fig. 66)

Synonyms: *Diplodinium unifasciculatum* Fiorentini, 1890; *Blepharocorys unifasciculatum* (Fiorentini, 1890) Sharp, 1915.

Specific diagnosis.—*Tetratoxum*: Seen from the side it is irregularly elliptical, with both ends rounded. The body is constant in form, rigid, noncontractile and about twice as long as it is thick. It is thickest at the middle. It is slightly flattened bilaterally.

The ectoplasm is a thin homogeneous layer. The endoplasm is coarsely granular and contains plant fibers and other large particles of food. The whole body is covered by a fairly thick, firm and refractive cuticle. There are longitudinal rows of 7-9 cuticular ridges on both the dorsal and ventral surfaces of the body. Lateral cuticular extensions at the posterior end form two caudal sheaths. These two caudal sheaths are not in direct opposition to each other; the right one is more ventral while the left one is more dorsal. There are also concentric ridges on the inner surfaces of these caudal sheaths.

The cytostome which is a ventral slit surrounded by short cilia is situated near to the anterior extremity of the body. Just behind the peristome there is the mental arch of membranelles which runs from the right side to the ventral side. The occipital arch of membranelles runs from the left side of the body near to the left end of the mental arch dorsally to the dorsal side a little below the right end of the mental arch. The dorsal caudal arch of membranelles occupies a slanting line from left to right on the dorsal surface at the posterior end of the body. The ventral caudal arch of membranelles occupies also a slanting groove from right to left on the ventral surface at the posterior end of the body. The cytoproct lies between the two caudal sheaths at the dorsal side of the right one. The anal canal is broad.

The macronucleus is coarsely granular. It is situated under the dorsal surface near to the right surface at the anterior part of the body. Its anterior end forms a short hook which directs ventrally. The body of the macronucleus which is about three times as long as the hook directs poster-



iad. There is usually a slender neck connecting the hook and the body of the macronucleus. A small refractive and more or less ellipsoidal micronucleus is found posterior to the neck and just ventral to the body of the macronucleus.

A large contractile vacuole is found under the curvature of the macronucleus. Reproduction is by transverse fission.

Measurements of 49 specimens yield the following results:

	Length	Thickness
Range .....	104 - 168 $\mu$	62 - 100 $\mu$
Mean .....	134.75 $\pm$ 1.35 $\mu$	76.16 $\pm$ 0.66 $\mu$
Standard deviation .....	14.17 $\mu$	6.98 $\mu$
Coefficient of variation ...	10.51 %	9.16 %

Habitat: colon.

Geographic distribution: Italy, Russia, U. S. A.

## 2. *Tetratoxum excavatum* sp. nov.

(Pl. V, Fig. 67)

Specific diagnosis.—*Tetratoxum*: Seen from the side this protozoon is also irregular elliptical in shape, with both ends rounded. The body is constant in form, rigid, noncontractile and about  $1\frac{1}{2}$  times as long as it is thick. It is thickest at the middle. Both dorsal and ventral surfaces are strongly convex. It is slightly flattened bilaterally. The ectoplasm is a thin homogeneous layer like that of the preceding species. The endoplasm is distinctly granular and contains plant fibers and also other large particles of food. The cuticle is a fairly thick, firm and refractive layer. At the anterior end on the right surface the body between the two anterior arches of membranelles there is a deep elliptical excavation covered by a flap of cuticle. This character differs from the preceding species. The cuticular ridges are more prominent and the adjacent ridges are farther apart than those of the preceding species. Like the preceding species the lateral cuticular extensions at the posterior end of the body form two caudal sheaths. There are also concentric ridges on the inner surfaces of these caudal sheaths.

The cytostome which is also a ventral slit guarded by short cilia is situated near the anterior extremity of the body. Both mental and occipital arches resemble those of the preceding species. The cytopye lies between the two caudal sheaths. It is connected by a broad anal canal.

The macronucleus is coarsely granular. It is sharply curved and in two parts. The anterior part is more massive and directed antero-ventrad. The posterior part is shorter and less massive and is directed posteriad. A small ellipsoidal micronucleus is situated just ventral to the angle of the macronucleus. A large contractile vacuole is to be found under the anterior part of the macronucleus.

Measurements of 25 specimens yield the following results:

	Length	Thickness
Range .....	95 - 135 $\mu$	55 - 90 $\mu$
Mean .....	115.76 $\pm$ 1.48	73.48 $\pm$ 1.46 $\mu$

Standard deviation .....	11.16 $\mu$	10.92 $\mu$
Coefficient of variation ...	9.64 %	14.86 %

Habitat: colon.

Geographic distribution: U. S. A.

### 3. *Tetratoxum parvum* sp. nov.

(Pl. V, Fig. 68)

Specific diagnosis.—*Tetratoxum*: Seen from the side it is also irregularly elliptical, with both ends rounded. The body is constant in form, rigid and noncontractile just as with the other species. It is a little less than twice as long as it is thick. It is thickest at the middle third of the body. It is also slightly flattened bilaterally but both dorsal and ventral surfaces are almost straight and parallel to each other.

The ectoplasm is a thin homogeneous layer. The endoplasm is coarsely granular and also contains plant fibers and other food particles. The cuticle is also a thin, firm and refractive layer. There are no cuticular ridges on both dorsal and ventral surfaces. Neither do the caudal sheaths show any markings.

The cytostome is a ventral slit guarded by cilia at the anterior part of the body. Both mental and occipital arches resemble those of the preceding species with respect to their arrangement and location. Both caudal arches also resemble those of the preceding species. The cytophyge and the anal canal are also in their usual positions.

The macronucleus is coarsely granular and resembles that of *T. excavatum* in general shape and location. The massive anterior portion bends antero-ventrally and the smaller posterior portion directs posteriad. A small ellipsoidal micronucleus is situated at the curvature of the macronucleus. The contractile vacuole is situated partly on the right side of the anterior portion of the macronucleus just anterior to the micronucleus. Reproduction is by transverse fission.

Measurements of 49 specimens yield the following results:

	Length	Thickness
Range .....	67 - 98 $\mu$	39 - 52 $\mu$
Mean .....	80.34 - 0.67 $\mu$	45.93 $\pm$ 0.34 $\mu$
Standard deviation .....	7.04 $\mu$	3.56 $\mu$
Coefficient of variation ...	8.76 %	7.75 %

Habitat: colon.

Geographic distribution: U. S. A.

Genus: *TEIPALMARIA* Gassovsky, 1919

Synonym: *Tricaudalia* Buisson. 1923.

Generic diagnosis.—Cycloposthiidae: Body with three caudalia, two dorsal and one ventral.

Type-species: *Tripalmaria dogieli* Gassovsky, 1919.

***Tripalmaria dogieli* Gassovsky, 1919**

(Pl. V, Figs. 69-70)

Specific diagnosis.—*Tripalmaria*: Seen from the side it is irregularly oval in shape. It is constant in form, rigid, noncontractile and about twice as long as it is thick. It is thickest in the middle. It is slightly flattened bilaterally. Both ends are more or less rounded. Its ventral side is fairly straight and at the posterior part in a slight depression is the ventral caudalium. The dorsal side is uneven. In a depression at the anterior part of the body is the anterior caudalium. The posterior dorsal caudalium is situated in another depression in the posterior part of the body opposite that of the ventral side. These two dorsal caudalia are separated from each other by elevations of the body.

The ectoplasm is a very thin homogeneous layer. The endoplasm is coarsely granular and contains food particles. The whole body is covered by a highly punctate cuticle. There are skeletal plates under the cuticle. On the right side of the body the skeletal plates are in the form of a horseshoe with the opening toward the posterior end. There are also longitudinal striations under the cuticle on the right side of the body. On the left side of the body only the dorsal part is supported by two articulated skeletal plates.

The peristome is at the anterior end, a little to the dorsal side. It is surrounded by a ring of adoral membranelles. The cuticle around the peristome is wrinkled forming numerous longitudinal ridges. The cytopygge is located on the dorsal side at the posterior end of the body, just back of the postero-dorsal caudalium. A much wrinkled anal tube can easily be observed.

The macronucleus is bilobed. Its ventral lobe which is constricted at the middle, is located partially under the ventral arm of the horseshoe-shaped skeletal plates. The narrow strip, which connects the dorsal and ventral lobes, arises from the anterior portion of the ventral lobe close to the cuticle of the right side and extends toward the dorsal side. At the dorsal side it turns under a skeletal plate and again it turns around the edge of the left skeletal plate in the form of a knife blade. A comparatively large ellipsoidal micronucleus is found at the constriction of the ventral lobe of the macronucleus. Just anterior to the micronucleus a contractile vacuole is also present. Reproduction is by transverse fission.

Measurements of 49 specimens yield the following results:

	Length	Thickness
Range .....	77 - 123 $\mu$	47 - 62 $\mu$
Mean .....	102.63 $\pm$ 0.96 $\mu$	53.24 $\pm$ 0.47 $\mu$
Standard deviation .....	10.12 $\mu$	4.98 $\mu$
Coefficient of variation ...	9.88 %	9.35 %

Habitat: colon.

Geographic distribution: Russia, U. S. A.

Genus: *COCHLIATOXUM* Gassovsky, 1919

Generic diagnosis.—Cycloposthiidae: Body with adoral and three other arches of membranelles, one occipital and two caudal.

Type-species: *Cochliatoxum periachtum* Gassovsky, 1919; only species of the genus.

***Cochliatoxum periachtum* Gassovsky, 1919**

(Pl. V, Fig. 71)

Specific diagnosis.—*Cochliatoxum*: It is more or less cylindrical in shape, with both ends rounded. It is rigid, noncontractile and about  $1\frac{1}{2}$  times as long as it is wide.

The ectoplasm is a thin homogeneous layer. The endoplasm is coarsely granular and contains plant fibers and other large food particles. The whole body is covered by a cuticle. The occipital arch of membranelles which is at the anterior half of the body extending spirally from the left side at the region just above the left corner of the peristome over to the middle of the right side, a little below the right corner of the peristome. The two caudal arches of membranelles which are at the posterior half of the body are two half spirals. The dorsal caudal arch extends spirally backward from the middle of the left side to the middle of the right side. The ventral caudal arch extends spirally from the right side over to the left at a point just a little below the dorsal arch.

The cytostome is a slit-shaped opening at the ventral side of the anterior end of the body. It is surrounded by two rows of adoral membranelles. The cytopyge is situated between the two caudal sheaths at the posterior end of the body. The much wrinkled anal tube is present.

The elongated macronucleus lies close to the right surface of the body. It is distinctly granular. The anterior and posterior thirds turn slightly toward the ventral side. The anterior end of the macronucleus forms a hook. The small ellipsoidal micronucleus is situated at the ventral side of the middle third of the macronucleus.

A contractile vacuole is usually found under the anterior third of the macronucleus. Reproduction is by transverse fission.

Measurements of 49 specimens yield the following results:

	Length	Thickness
Range .....	210 - 370 $\mu$	130 - 210 $\mu$
Mean .....	$273.36 \pm 3.62 \mu$	$163.67 \pm 1.75 \mu$
Standard deviation .....	36.86 $\mu$	18.32 $\mu$
Coefficient of variation ...	13.84 %	11.18 %

Habitat: colon.

Geographic distribution: Russia, U. S. A.

Genus: *DITOXUM* Gassovsky, 1919

Generic diagnosis.—Cyclopостиidae: In addition to adoral membranelles the body is provided with two dorsal arches, occipital and caudal.

Type-species: *Ditoxum funinucleum* Gassovsky, 1919; only species of the genus.



**Ditoxum funinucleum** Gassovsky, 1919  
(Pl. V, Fig. 72)

Specific diagnosis.—*Ditoxum*: Seen from the side it is elliptical, with both ends rounded. The body is constant in form, rigid and noncontractile and about twice as long as it is thick. It is slightly flattened bilaterally.

The ectoplasm is a thin, homogeneous layer. The endoplasm is coarsely granular and contains plant fibers and other large food particles. The whole body is covered by a firm cuticle. Posterior extension of the cuticle forms a caudal sheath. The main occipital arch of membranelles is located dorsally at the anterior end of the body, but it extends to both sides. The caudal arch of membranelles is also located on the dorsal side at the posterior end of the body and it extends only to the right side.

The peristome is on the ventral side at the anterior end of the body. It is surrounded by two rows of adoral membranelles. Two cytophyge is situated under the caudal sheath. The much wrinkled anal tube can easily be seen.

The macronucleus, which resembles that of *Tetratoxum unifasciculatum* except that it is a little larger posteriorly, lies under the dorsal side close to the right surface of the body. The small ellipsoidal micronucleus lies under the middle part of the macronucleus. A contractile vacuole is usually found just anterior to the micronucleus. Reproduction is by transverse fission.

Measurements of 49 specimens yield the following results:

	Length	Thickness
Range .....	135 - 203 $\mu$	70 - 101 $\mu$
Mean .....	171.28 $\pm$ 1.41 $\mu$	82.79 $\pm$ 0.57 $\mu$
Standard deviation .....	14.82 $\mu$	6.00 $\mu$
Coefficients of variation ...	8.65 %	7.24 %

Habitat: colon.

Geographic distribution: Russia, U. S. A.

Class: SUCTORIA Claparède and Lachman, 1858

Genus: *ALLANTOSOMA* Gassovsky, 1919

Generic diagnosis.—Body somewhat cycloid in shape and bearing one or more tenacles upon each extremity; both macronucleus and micronucleus present; single contractile vacuole; ciliated stages unknown.

Type-species: *Allantosoma intestinalis* Gassovsky, 1919.

*Key to the Species of Allantosoma*

1. More than one tenacle upon each extremity ..... *A. intestinalis*  
Only one tenacle upon each extremity ..... 2
2. Tenacle long, distal end boot-shaped ..... *A. dicorniger*  
Short tenacle, distal end rounded ..... *A. brevicorniger*

1. *Allantosoma intestinalis* Gassovsky, 1919

(Pl. V, Fig. 73)

Specific diagnosis.—*Allantosoma*: Body sausage-shaped, bearing 3 to 12 tenacles upon each extremity of the body. The suckers are distinct. The cytoplasm is filled with small round bodies. The macronucleus is more or less spherical and is usually situated in the center of the body. A spherical micronucleus lies by the side of the macronucleus. The body length varies from 33-60  $\mu$ , with a mean of 47.7  $\mu$ ; the width varies from 18-37  $\mu$ , with a mean of 26  $\mu$ . Specimens attached to *Cycloposthium bipalmatum*, *Blepharocorys curvigula*, *Blepharocorys uncinata* and *Blepharoprosthium pireum* have been observed.

Habitat: cecum and colon.

Geographic distribution: Russia, U. S. A.

2. *Allantosoma dicorniger* Hsiung, 1928

(Pl. V, Fig. 74)

Specific diagnosis.—*Allantosoma*: Body is more or less cycloid in shape, bearing one incurved tenacle on each end. The outline of the distal end of the tenacle somewhat resembles that of a boot. The surface lying between these two tenacles is nearly flat, while the remaining surface is convex. The cytoplasm is filled with numerous granules.

A subspherical macronucleus lies near the center of the body. A small micronucleus lies at the side of the macronucleus. A single contractile vacuole can usually be seen near the macronucleus. Body length varies from 20-33  $\mu$ , with a mean length of 27  $\mu$ ; the width varies from 10-20  $\mu$ , with a mean of 16.4  $\mu$ .

Every individual observed was unattached to any other organism.

Habitat: colon.

Geographic distribution: U. S. A.

3. *Allantosoma brevicorniger*, Hsiung, 1928

(Pl. V, Fig. 75)

Specific diagnosis.—*Allantosoma*: Body resembles that of an elongated *A. dicorniger*. It also bears one slightly incurved tenacle on each end of the body. The tenacles are short and slender. The distal end of the tentacle is rounded. The cytoplasm is slightly granular.

The ellipsoidal macronucleus lies in the center of the body. A small subspherical micronucleus lies at one end of the macronucleus. A single contractile vacuole is usually located near the macronucleus. The body length varies from 23-36  $\mu$ , with a mean of 29.6  $\mu$ ; the width varies from 7-11  $\mu$ , with a mean of 8.6  $\mu$ .

Some have been found attached to the body of *Didesmis ovalis*, *Paraisotricha colpoidea*, *Paraisotricha beckeri*, *Paraisotricha minuta* and *Blepharocorys jubata*.

Habitat: cecum and colon.

Geographic distribution: U. S. A.

## PHYSIOLOGICAL RELATIONSHIPS

Gruby and Delafond (1843) had the idea that those protozoa which grew upon plant materials in the large intestine of the horse really supplied animal protein to the herbivore by being digested by the host.

Bundle (1895) thought that these protozoa might assist in maceration and mixing of the cecal contents. He also had the idea that they might also help in transforming indigestible food material into easily digestible material.

After some experiments Bienenstein (1926) concluded that those protozoa played a very important rôle as a source of protein by converting indigestible food material into easily digestible animal protoplasm.

Fantham (1921) observed *Cycloposthium bipalmatum* in the feces of colicky horses. Counts were made by him daily. He stated, "Coincident with the increase in the number of the ciliates there was an increase in the softness and pulpiness of the feces of the horses. When the number of ciliates was very low or zero, the feces were formed and hard. As the number increased, the feces became softer and looser until at periods of maximum crop of ciliates the feces were practically diarrhoeic in character." It seems natural to the writer that loose feces from any number of causes would flush out a great number of protozoa, but when the feces are hard, most protozoa would become partially desiccated and destroyed. Therefore, Fantham's observations of themselves do not justify any assumption that these protozoa caused the colic of the horse.

Recently Becker, Schulz and Emmerson (1930) have proved by experiments that rumen infusoria are in no way a significant help to their host. They regard these protozoa as commensals. Although there is not yet a single experiment to support him, the writer is of the opinion that those protozoa in the large intestine of the horse are likewise harmless commensals.

## INFECTION

Bundle (1895) concluded that the horse got its infection of infusoria through *Dauerzustände* or cysts. Fantham (1921) traced the source of infection which caused colic of the horse to a dirty stable broom used in the infected stable. However, he did not state whether it is transmitted by cysts or by trophozoites. The writer has examined fecal material from six horses and material from the large intestine of 40 horses, but not once has he found any cysts. Instead, only living trophozoites are found. It is the writer's opinion that the infection is transmitted by the contamination of food and drink with feces containing living trophozoites.

Becker and Hsiung (1929) attempted to infect the rumen of an azoic goat with infusoria from the colic contents and fecal material of horses. In the first trial 10 c.c. of fluid from mixed materials of No. 22 and No. 23 were given through a stomach tube. A few days later 5 c.c. of each No. 22 and No. 23 were given in the same way. About two weeks after the second trial 5 c.c. of No. 23 were given again. Two weeks after the third trial 10 c. c. of No. 24 were given.

They identified in the material from the horses living specimens of *Cycloposthium bipalmatum*, *C. scutigerum*, *Blepharocorys angusta*, *B. curvignola*, *B. jubata*, *Didesmis quadrata*, *D. ovalis*, *Bundleia postciliata*, *Tripalmaria dogieli*, *Triadinium caudatum*, *T. galea*, *T. minimum*, *Ditoxum*

*funinucleum*, *Tetratoxum unifasciculatum*, *Spirodinium equi*, *Charon equi*, *Paraisotricha colpoidea* and *Allantosoma intestinalis*. Although they examined the rumen contents for weeks, no infection took place. This shows that the horse infusoria will not develop in the stomach of ruminants and that the horse infusoria are specific.

# SUMMARY

1. Of the 46 horses examined, four classes of protozoa,—Rhizopoda, Mastigophora, Ciliata and Suctorina, were represented in the fauna of the large intestine of the horse. The order Coccidia was not represented. The ciliates are by far the predominating class, both in numbers and in variety of forms.
2. Of the Rhizopoda, two species of the same genus were recorded. Of the Mastigophora, four species, two of which are new, were recorded. Of the Ciliata, forty-eight species, seven of which are new, belonging to twenty-four genera were recorded. Of the Suctorina, three species of the same genus were recorded.
3. All the oligotrichs, except Cycloposthium, were found only in the colon, while the other ciliates, except a few rare ones, were found in both cecum and colon.
4. The physiological importance of these protozoa to their host is still unsolved. It is suggested that they are mere commensals.
5. It is suggested that the infection is transmitted through trophozoites in the feces which contaminate the food and drink of other horses. Infection of the goat's rumen with horse infusoria was not successful.

# LITERATURE CITED

- BECKER, E. R. AND HSIUNG, T. S.  
1929. The method by which ruminants acquire their fauna of infusoria, and remarks concerning experiments on the host-specificity of these protozoa. Proc. Nat. Acad. Sci., 15:684-690.
- BECKER, E. R., J. A. SCHULZ, AND M. A. EMMERSON  
1930. Experiments on the physiological relationships between the stomach infusoria of ruminants and their hosts, with a bibliography. Iowa State College Jour. Sci., 4:215-251.
- BIENERT, G.  
1926. I. Mitteilung: Über die Stickstoffverteilung im Coecum-inhalt des Pferdes mit besonderer Berücksichtigung der auf die Mikroorganismen entfallenden N-Menge. Im: Ueber die ernährungsphysiologische Bedeutung der Mikroorganismen im Darmtrakt der Pflanzenfresser von Carl Schwarz. Pfluegers Arc. f. d. Gesam. Phys. d. Mensch. u. d. Tiere, 213:556-562.
- BUISSON, J.  
1923. Les infusories ciliés du tube digestif de l'homme et des mammifères. Paris, Legrand. (Travail du Laboratoire de Parasitologie de la Faculté de Médecine de Paris, Paris), 200 pp. 60 figs. in text.  
1923. Sur quelques infusories nouveaux ou par connus parasites des mammifères. Ann. Parasitol., 1:209-246.
- BUNDLE, A.  
1895. Ciliate Infusorien im Coecum des Pferdes. Zeitschr. f. wiss. Zool. 60:284-350.
- COLIN, G.  
1871. Traité de physiologie comparée des animaux domestiques. 2nd edition, Paris, Baillière, p. 836-837.



- CUNHA, A. da  
1917. Sobre a presenca do *Balantidium* no cavallo. *Brazil-Medico*, **31**:337.  
1917. Sobre os ciliados do tubo digestivo dos mamiferos. *Buenos Aires*, 1-8.  
1919. Cilies intestinaux des mammiferes. *Mem. Inst. Oswaldo Cruz*, **11**:1-5.
- CUNHA, A. DA ET MUNIZ, J.  
1928. Nouveau Cilié, parasite du cæcum du *Tapirus americanus*. Description d'un nouveau genre. *C. R. Soc. Biol.* **98**:631-632.
- DOFLEIN, F. AND REICHENOW, E.  
1929. *Lehrbuch der Protozoenkunde*. 5. Aufl. Gustav Fisher, Jena, viii + 1262, text-figs. 1201.
- DOGIEL, V. A.  
1929. Die sog. "Konkrementenvakuole" der Infusorien als eine Statocyste betrachtet. *Arch. f. Protistenk.*, **68**:319-348.
- FANTHAM, H. B.  
1921. Some parasitic protozoa found in South Africa: IV. The South Africa Jour. Sci., **18**:164-170.
- FIORENTINI, A.  
1890. Intorno ai protisti dell'intestino degli equini. *Boll. Sci., Pavia*, **12**:7-17, 51-60.
- FONSECA, O. O. R. da  
1916. Estudo sobre on flagellados parasitos dos mamiferos do Brazil. *Mem. Inst. Oswaldo Cruz*, **8**:5-40.
- GASSOVSKY, G.  
1919. Notes et Communications. On the microfauna of the intestine of the horse. *Trav. de la Soc. des Natur. de Pétrograd* (1918), **49**:20-37.
- GEDOELST, L.  
1911. Synopsis de Parasitogie de l'homme et des animaux domestiques Lierre. Bruxelles, xx + 332 pp. text-figs. 327.
- GRUBY ET DELAFOND.  
1843. Recherches sur de animalecules se developpant en grand nombre dans l'estomac et dans les intestins, pendant la digestion des animaux herbivores et carnivores. *Compt. Rend. Acad. Sci., Paris*, **17**:1304-1308.
- HICKSON, S. J.  
1903. The Infusoria or Corticata Heterokaryota. In: *A Treatise on Zoology* by E. Ray Lankester, Part I:361-426, text-figs. 97.
- HSIUNG, T. S.  
1928. Suctoria of the large intestine of the horse: *Allantosoma intestinalis* Gassovsky, *A. dicorniger* sp. nov., and *A. brevicorniger* sp. nov. *The Iowa State College Jour. Sci.*, **3**:101-103.  
1929. On *Didesmis spiralis* sp. nov., a new ciliate from the large intestine of the horse. *Trans. Amer. Micros. Soc.*, **48**:209-213.  
1929. A survey of the protozoan fauna of the large intestine of the horse. *Jour. Parasit.* **16**: (Abs.) p. 99.  
1930. Some new ciliates from the large intestine of the horse. *Trans. Amer. Micros. Soc.*, **49**:34-41.
- JAMESON, A. P.  
1925. A new ciliate, *Charon ventriculi* n. g., n. sp., from the stomach of ruminants. *Parasitology*, **17**:403-405.
- POCHE, F.  
1913. Das System der Protozoa. *Arch. f. Protistenk.*, **30**:125-321.

SHARP, R. G.

1914. *Diplodinium ecaudatum*, with an account of its neuromotor apparatus. Univ. of Calif. Pub. in Zool., 13:43-122.

SCHUMACHER, I. C.

1915. On *Blepharocorys equi* n. sp., a new ciliate from the caecum of the horse. Univ. Calif. Pub. in Zool., 16:95-106.

STRELKOW, A.

1928. Nouvelles espèces du genre *Cycloposthium* habitant l'intestin du cheval. Ann. Parasitol., 6:164-178.

1929. Weiteres über die neuen Arten der Gattung *Cycloposthium* aus dem Darne des Pferdes und des Esels. Zool. Anz., 83:63-70.

1929. Morphologische Studien über oligotriche Infusorien aus dem Darne des Pferdes. Arch. f. Protistenk., 68:503-554.

WEISS, D. C. F. H.

1869. Specielle Physiologie der Haussäugethiere für Thierärzte und Landwirthe. Stuttgart, Metzler, xii + 548 pp., text-figs. 88

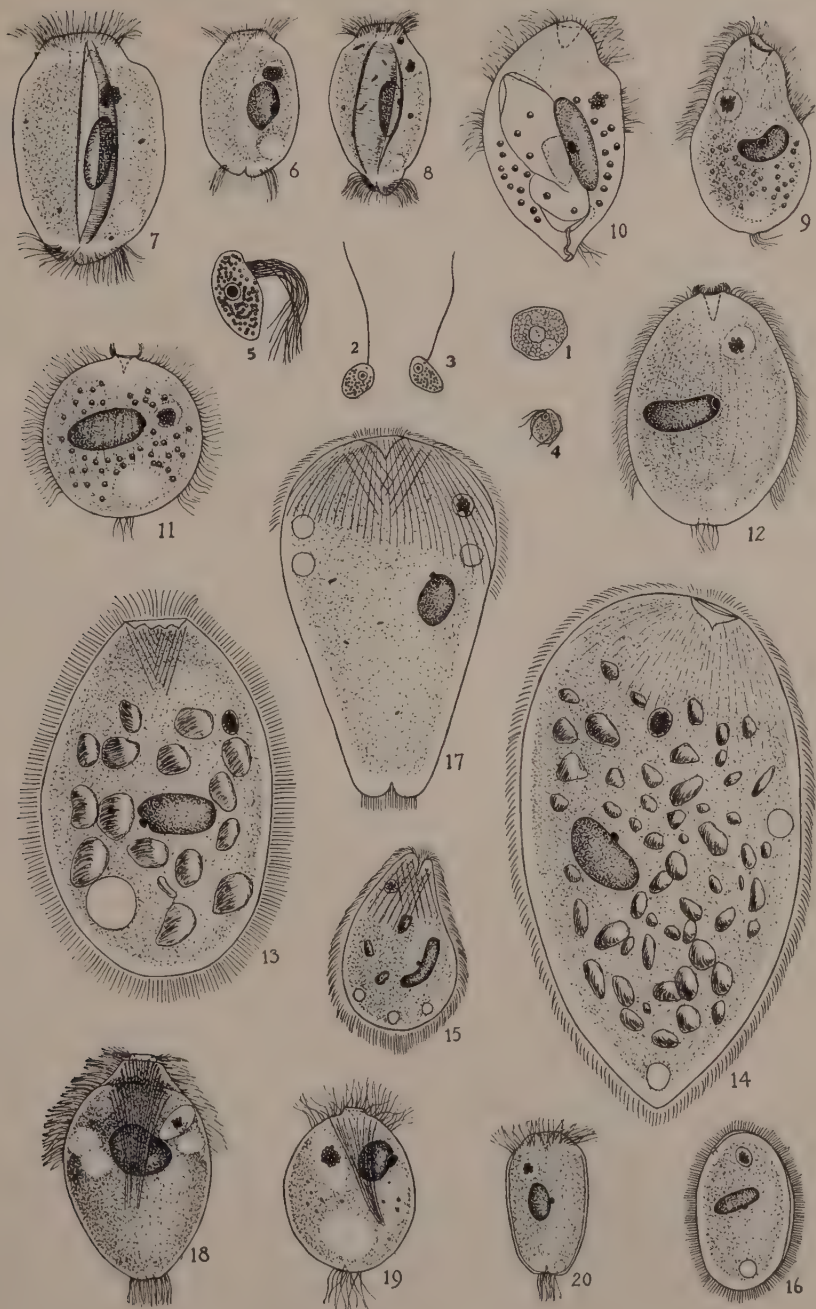
WENYON, C. M.

1926. Protozoology. William Wood and Co., New York. xvi + 1563 pp., 565 figs. and 20 colored plates.

## PLATE I

(All drawings are original except when stated otherwise.)

- Fig. 1. *Endamoeba gedoelsti*. X 833.  
Figs. 2-3. *Oikomonas equi*. X 833.  
Fig. 4. *Trichomonas equi*. X 833.  
Fig. 5. *Callimastix equi*. X 833.  
Fig. 6 Dorsal view of *Didesmis ovalis*. X 403.  
Fig. 7. Dorsal view of *Didesmis quadrata*. X 403.  
Fig. 8. Dorsal view of *Didesmis spiralis*. X 403.  
Fig. 9. *Blepharoprosthium pireum*. X 403.  
Fig. 10. A specimen of *B. pireum* digesting *Blepharocorys uncinata*. X 403.  
Fig. 11. *Blepharosphaera intestinalis*. X 403.  
Fig. 12. *Blepharosphaera ellipsoidalis*. X. 403.  
Fig. 13. *Holophryoides ovalis*. X 403. (After Gassovsky, 1919.)  
Fig. 14. *Blepharozoum zonatum*. X 400. (After Gossovsky, 1919.)  
Fig. 15. *Prorodonopsis coli*. X 403. (After Gassovsky, 1919.)  
Fig. 16. *Paraisotrichopsis composita*. X. 403. (After Gassovsky, 1919.)  
Fig. 17. *Blepharoconus hemiciliatus*. X 403. (After Gassovsky, 1919.)  
Fig. 18. *Blepharoconus cervicalis*. X 403. (After Hsiung, 1930.)  
Fig. 19. *Blepharoconus benbrooki*. X 833.  
Fig. 20 *Bundelia postciliata*. X 403.





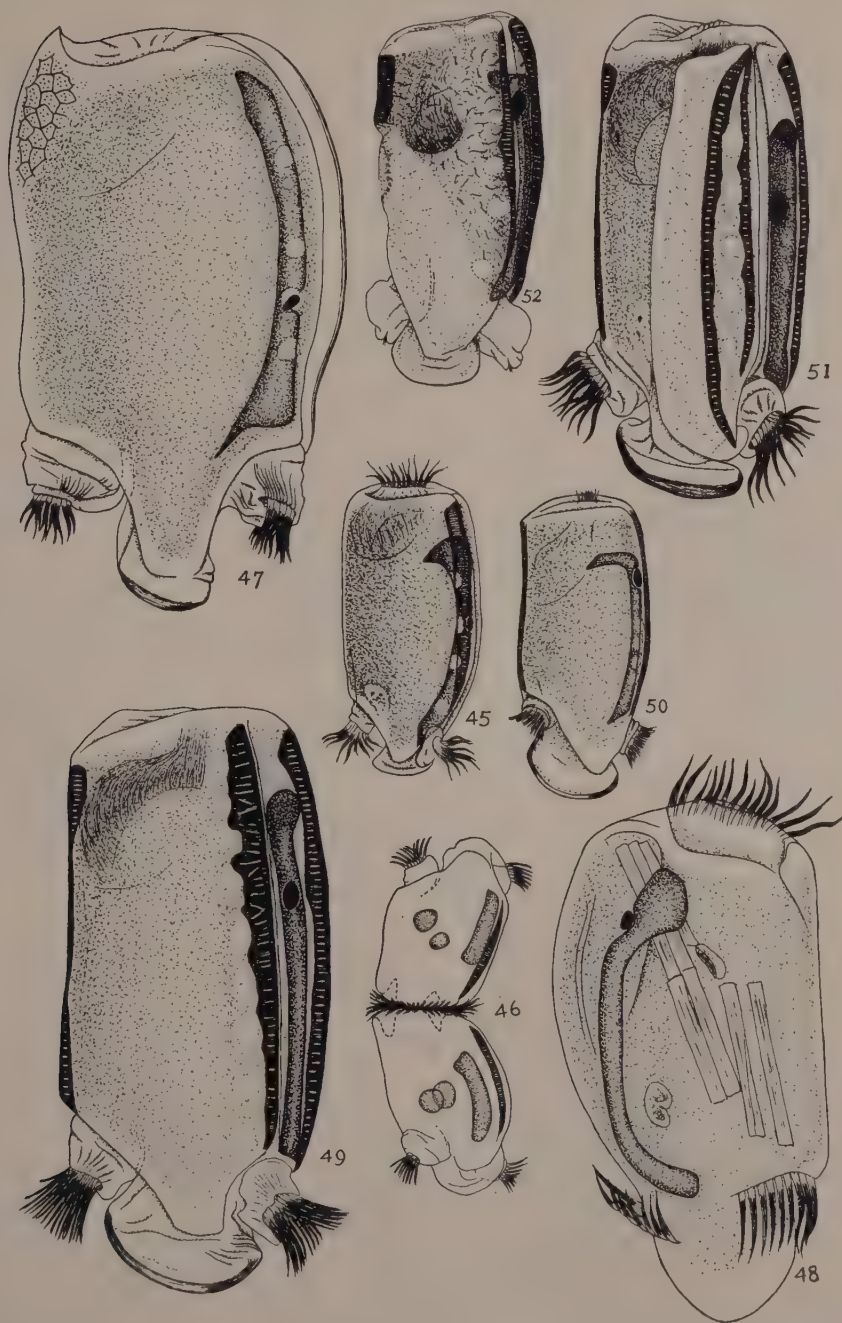
## PLATE II

- Fig. 21. *Alloiozona trizona*. X 403. (After Hsiung, 1930.)  
Fig. 22. A specimen of *A. trizona* parasitized by *Sphaerita*. X 403.  
Fig. 23. *Polymorpha ampulla*. X 833.  
Fig. 24. *Ampullacula ampulla*. X 403. (After Fiorentini, 1890.)  
Fig. 25. *Blepharocodon appendiculatus*. X 833. (After Bundle, 1895.)  
Fig. 26. Lateral view of *Paraisotricha colpoidea*. X 403.  
Fig. 27. Lateral view of *Paraisotricha beckeri*. X 403. (After Hsiung, 1930.)  
Fig. 28. Lateral view of *Paraisotricha minuta*. X 403.  
Fig. 29. A specimen of *P. minuta* parasitized by *Sphaerita*. X 403.  
Fig. 30. A specimen of *P. minuta* parasitized by *Allantosoma brevicorniger*. X 403.  
Figs. 31-32. Exconjugants of *P. minuta*. X 403.  
Fig. 33. Lateral view of *Blepharocorys uncinata*. X 403.  
Figs. 34-38. Specimens of *B. uncinata* showing the development of the anterior process. X 403.  
Fig. 39. Lateral view of *Blepharocorys valvata*. X 403.  
Fig. 40. Lateral view of *Blepharocorys jubata*. X 403.  
Fig. 41. Lateral view of *Blepharocorys curvigula*. X 403.  
Fig. 42. Lateral view of *Blepharocorys angusta*. X 403.  
Fig. 43. Lateral view of *Blepharocorys cardionucleata*. X 403.  
Fig. 44. Dorsal view of *Charon equi*. X 833. (After Hsiung, 1930.)



## PLATE III

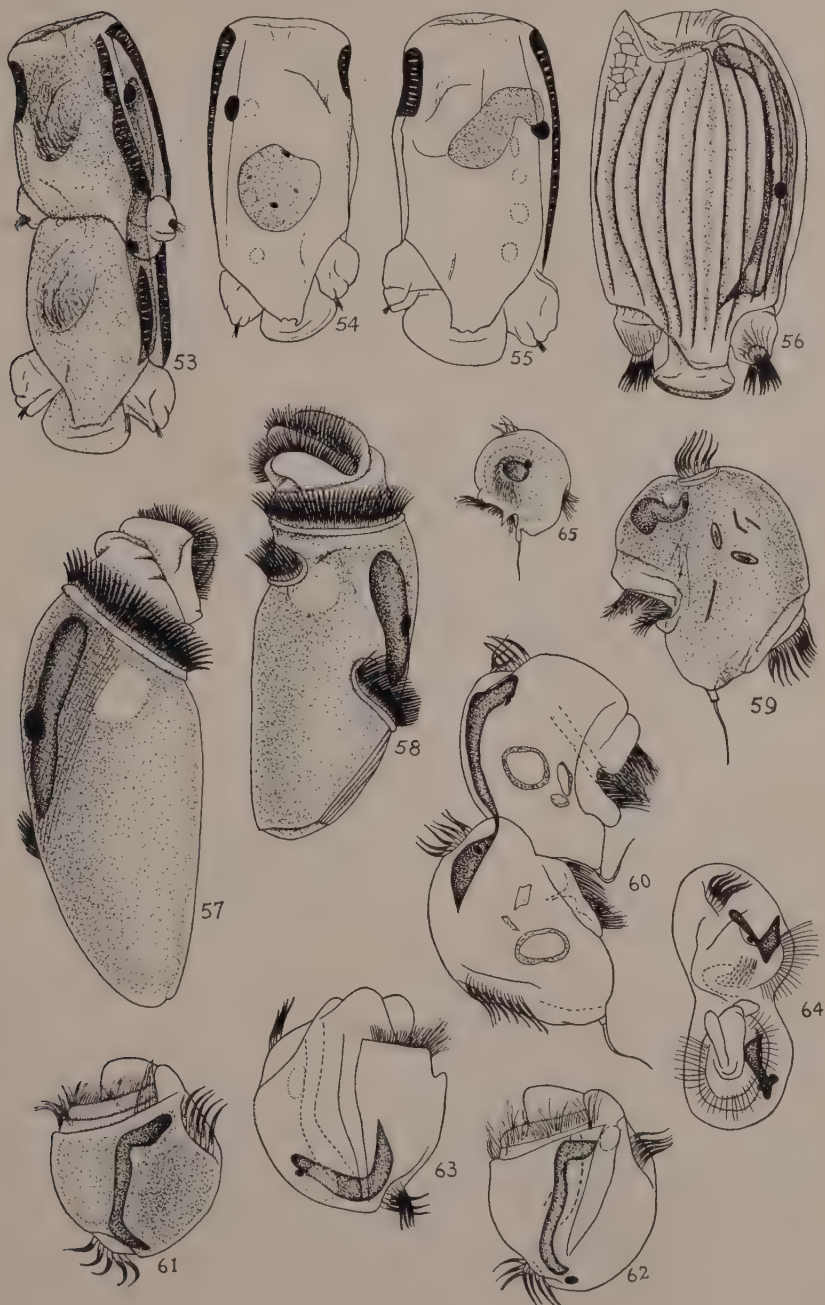
- Fig. 45. Lateral view of *Cycloposthium bipalmatum*. X 403.  
Fig. 46. Conjugating specimens of *C. bipalmatum*. X 403.  
Fig. 47. Lateral view of *Cycloposthium dentiferum*. X. 403.  
Fig. 48. Lateral view of *Cycloposthium ishikawai*. X 290. (After Gassovsky, 1919.)  
Fig. 49. Lateral view of *Cycloposthium edentatum*. X 403.  
Fig. 50. Lateral view of *Cycloposthium piscicauda*... X. 290. (After Strelkow, 1928 from Annales de Parasitologie. Modified.)  
Fig. 51. Lateral view of *Cycloposthium scutigerum*. X 403.  
Fig. 52. Laterval view of *Cycloposthium affinae*. X 403.





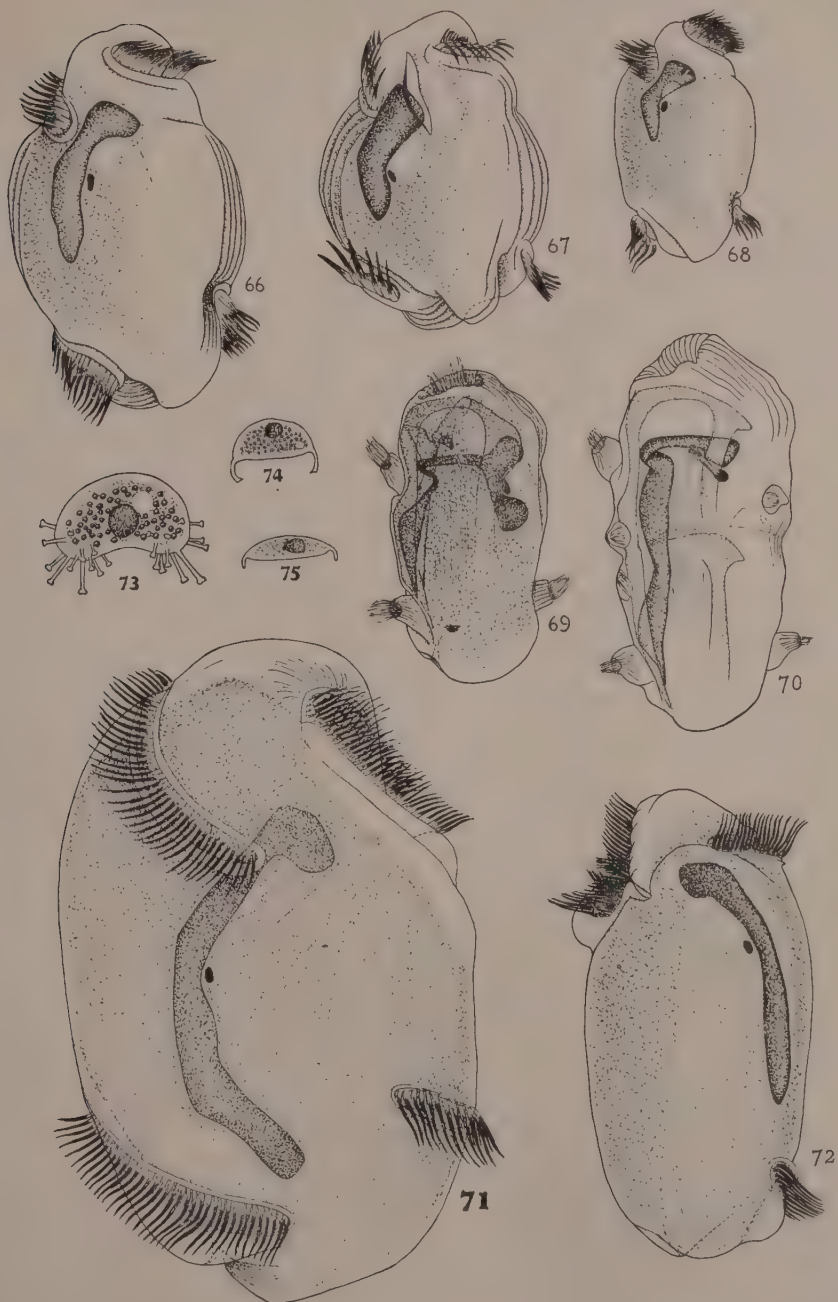
## PLATE IV

- Fig. 53. Dividing form of *C. affinae*. X 403.  
Figs. 54-55. Exconjugants of *C. affinae*. X 403.  
Fig. 56. Lateral view of *Cycloposthium corrugatum*... X 403.  
Figs. 57-58. Lateral views of *Spirodinium equi*. X 403.  
Fig. 59. Lateral view of *Triadinium caudatum*. X 403.  
Fig. 60. Dividing form of *T. caudatum*. X 403.  
Fig. 61. Lateral view of *Triadinium galea*. X 403.  
Figs. 62-64. Dividing forms of *T. galea*. X 403.  
Fig. 65. Lateral view of *Triadinium minimum*. X 403.



## PLATE V

- Fig. 66. Lateral view of *Tetratoxum unifasciculatum*. X 403.  
Fig. 67. Lateral view of *Tetratoxum excavatum*. X 403.  
Fig. 68. Lateral view of *Tetratoxum parvum*. X 403.  
Fig. 69. Lateral view of *Tripalmaria dogieli*. X 403.  
Fig. 70. Divising form of *T. dogieli*. X 403.  
Fig. 71. Lateral view of *Cochliatoxum periachtum*. X 403.  
Fig. 72. Lateral view of *Ditoxum funinucleum*. X 403.  
Fig. 73. *Allantosoma intestinalis*. X 403.  
Fig. 74. *Allantosoma dicorniger*. X 403.  
Fig. 75. *Allantosoma brevicorniger*. X 403.







## STUDIES ON NITROGEN FIXATION IN SOME IOWA SOILS

R. H. WALKER AND P. E. BROWN\*

*From the Laboratory of Soil Bacteriology, Agricultural Experiment Station,  
Iowa State College*

Accepted for publication April 5, 1930

The fact that nitrogen may be added to field soils through the activities of microorganisms was first established by Berthelot. His attempts to isolate the causative agents in this nitrogen fixation process were unsuccessful, but a few years later Winogradsky succeeded in isolating an anaerobic spore forming organism which possessed this ability. He named this organism *Clostridium pasteurianum*. In 1901 Beijerinck isolated another type of organism which was able to fix atmospheric nitrogen in the soil. This was an aerobic organism and it did not produce spores. He gave it the name *Azotobacter chroococcum*. Other species belonging to each of these genera have since been discovered and likewise have been found to be capable of fixing atmospheric nitrogen.

Since the discovery of these nitrogen-fixing bacteria many researches have dealt with their distribution in soils and their economic importance in the maintenance of soil fertility. These bacteria are widely distributed in nature, having been found in many soil types from practically all nations of the world. There are some definite limits to their distribution, however. This is especially true of the aerobic group of organisms of the genus *Azotobacter*.

It has long been known that the organisms of this genus are sensitive to soil acidity, and it has been shown by Gainey (1), Niklas, Poschenrieder and Hock(2), and others, that these organisms very seldom occur or that they are inactive in soils with an acidity greater than pH 6.0. A few soils more acid than pH 6.0 were found to contain *Azotobacter* but the majority did not. Many soils with less acidity than pH 6.0 were also found to be without these organisms and in some cases where the organisms were present they did not appear to be vigorous and were considered to have a low physiological efficiency with respect to nitrogen fixation. Practically all the studies on the occurrence and distribution of the aerobic nitrogen-fixing bacteria, and especially those belonging to the genus *Azotobacter*, have indicated that these organisms are very sensitive to environmental conditions. They seem to be especially sensitive toward soil acidity. In addition, their growth and nitrogen-fixing ability are affected by other soil deficiencies, and particularly by a lack of available phosphorus. In fact their growth and nitrogen-fixing ability are limited to such an extent by deficiencies of lime and phosphorus in soils that a number of so-called *Azotobacter* tests have been devised to test soils for deficiencies in these two constituents.

---

\*Assistant Chief and Chief in Soil Bacteriology.

The anaerobic nitrogen-fixing bacteria of the *Clostridium* group have been found to be more generally distributed in nature. They have been found in practically all soils tested, including soils of many countries and of widely varying formation and characteristics. They are not as sensitive to soil acidity as the aerobic *Azotobacter* organisms, and apparently they are not affected as much by other soil deficiencies.

The question of the relative economic importance of these two groups of nitrogen-fixing bacteria in soils is an extremely interesting one. Ever since the discovery of the aerobic organisms they have been considered to be of the greatest economic importance in cultivated soils. But in spite of this belief, it has been known that the aerobic organisms are entirely absent in many soils and relatively inefficient in others. In such soils this group of organisms can be of no economic importance. On the other hand, although the anaerobic organisms may be present in most soils, it has been considered by many that they were relatively unimportant except in such abnormal soils as those that were poorly drained and unsuited for the economic production of crops. In recent years, however, some investigators have been led to believe that the anaerobic nitrogen-fixers are of greater importance in adding nitrogen to soils than has generally been considered possible. Upon this point there has been no conclusive evidence, and it seems that an investigation into the question of the actual importance of these two groups of nitrogen-fixing organisms in maintaining the nitrogen supply of field soils would be very desirable. In this connection, some interesting points have been brought out in our studies on nitrogen fixation during the past year. The results which are presented here are by no means conclusive but merely emphasize the need of a thorough study of the relative importance of these two groups of bacteria in field soils.

In one of our experiments where various kinds and amounts of lime had been applied to Grundy silt loam, tests are being conducted to determine the effect of the various lime treatments upon the bacteriological activities in the soil. The Grundy silt loam is a loessial or wind blown soil; it is fairly rich in organic matter, and it has a high buffering capacity but is quite acid, the pH being about 5.4 to 5.6.

Among other tests on this soil the nitrogen-fixing power of the treated soils was measured. Two methods were outlined for this study. First, it was planned to measure the amount of nitrogen fixed when one gram of fresh soil was used to inoculate a silica gel medium in large petri dishes, which were subsequently allowed to incubate for 7 days at 28°C. Second, it was planned to measure the amount of nitrogen fixed when five grams of fresh soil were used to inoculate 50cc. of a nitrogen-free mannite solution, which was then incubated for 3 weeks at 28°C.

The results of these studies are presented in table 1. These data show that there was no fixation of nitrogen on the silica gel. But there was a definite fixation of nitrogen in the solution cultures when the same soils were used for inocula. Two things may be assumed from these data: either the silica gel medium may not be suitable for the growth of nitrogen-fixing bacteria, or the two media present such widely different environments that certain groups of organisms which will grow on one will not develop on the other.

TABLE 1. *Milligrams of Nitrogen Fixed on Silica Gel and in Solution Culture when Inoculated with Grundy silt loam*

Plot no.	Soil treatment	Milligrams of nitrogen fixed	
		Silica gel inoc. with one gram of soil	Solution culture inoculated with five grams of soil
1	No lime	-0.08	5.66
2	One ton of lime	0.14	6.82
3	Two tons of lime	-0.52	7.39
4	Three tons of lime	-0.59	6.50
5	Four tons of lime	0.27	7.01
6	Five tons of lime	-0.01	5.91
7	Six tons of lime	0.14	6.39
8	No lime	0.18	4.55
9	20 mesh lime, 3 tons	0.14	6.52
10	40 mesh lime, 3 tons	-0.12	6.80
11	100 mesh lime, 3 tons	-0.38	6.31
12	Hydrated lime, equivalent to three tons lime	-0.13	7.55

In order to clarify the first point, tests were conducted using soils from another set of experimental plots, some of which were known to support a vigorous growth of *Azotobacter* and others in which no *Azotobacter* have been found. This series of experimental plots is located on the Carrington loam. The soil treatments and results secured in these nitrogen-fixation tests are presented in table 2.

TABLE 2. *Milligrams of Nitrogen Fixed on Silica Gel when Inoculated with Carrington Loam*

Plot no.	Soil treatment	pH of soil	Milligrams of nitrogen fixed on silica gel
905	No treatment	5.60	11.97
906	Manure	5.98	12.14
907	Manure + lime	7.11	11.05
908	Lime	6.23	10.57
909	No treatment	5.57	0.54

These results show that the silica gel medium is suitable for nitrogen fixation measurements. In fact, there was about twice as much nitrogen fixed in these experiments on Carrington loam as there was in the previous experiments with Grundy silt loam. Inasmuch as a different soil was used in the two experiments another experiment was conducted as follows: portions of soil from plots 1, 4, and 12 of the Grundy silt loam experiment were inoculated with a pure culture of *Azotobacter* and allowed to incubate for a few days, after which one gram of soil was used to inoculate silica



gel plates. To serve as controls, other plates were inoculated with soils which had not been inoculated with a pure culture of *Azotobacter*. In addition, other silica gel plates were inoculated with a pure culture of *Azotobacter* without soil. The results secured in these tests are shown in table 3. These data show that the pure culture of *Azotobacter* fixed appreciable amounts of nitrogen on silica gel plates under the conditions of the experiment. They also show that large amounts of nitrogen were fixed on the silica gel plates when inoculated with soils that were known to contain *Azotobacter*, even when these soils gave negative results in nitrogen fixation tests under similar conditions before *Azotobacter* organisms were added to them.

TABLE 3. *Milligrams of Nitrogen Fixed on Silica Gel Plates when inoculated with a pure culture of Azotobacter and when inoculated with Grundy silt loam with and without Azotobacter*

Soil no.	Soil treatment	Milligrams of nitrogen fixed on silica gel plates
1	Not inoculated	0.55
1	Inoculated with <i>Azotobacter</i>	10.03
4	Not inoculated	0.63
4	Inoculated with <i>Azotobacter</i>	9.56
12	Not inoculated	0.18
12	Inoculated with <i>Azotobacter</i>	10.17
<i>Azotobacter</i> without soil		7.07
<i>Azotobacter</i> without soil		7.65

It would seem then, that no nitrogen fixation occurred in the original tests on Grundy silt loam because of a lack of aerobic nitrogen-fixing organisms. When these organisms were added to the soil an appreciable nitrogen fixation took place.

On the other hand, appreciable amounts of nitrogen were fixed in solution cultures when inoculated with the Grundy soils. It seems that the difference in results secured with the two methods for the Grundy soils may be due to a fixation of nitrogen by the anaerobic bacteria of the soil. This has not been proven definitely but has been assumed for the following reasons. On the silica gel plates the growth conditions were undoubtedly favorable for the growth of aerobic nitrogen fixing organisms but unfavorable for the anaerobic organisms. In the solution cultures, however, the growth conditions may have been favorable at least partially for the anaerobic organisms, thus permitting of their growth and the fixation of nitrogen.

Whether the anaerobic organisms are active in fixing atmospheric nitrogen in these soils in the field is not known; and whether or not they would add nitrogen to the soil in the field if the aerobic *Azotobacter* organisms were present, is likewise unknown. Neither do we know how much nitrogen either of these groups of bacteria will add to field soils in the course of a year, nor the relative importance of each group. It seems

that these questions warrant a thorough investigation and that their answering would be of practical importance as well as of much interest from the scientific point of view.

## LITERATURE CITED

1. GAINNEY, P. L.  
1927. The occurrence of *Azotobacter* in soil. *Proc. First Int. Con. Soil Sci.*,  
3:107-117.
2. NIKLAS, H., H. POSCHENRIEDER, AND A. HOCK  
1926. Über die Verbreitung des *Azotobacters* in den Boden Bayerns unter  
Berücksichtigung der Bodenreaktions, des Kalk—und Phosphorsauregehaltes  
derselben. *Centbl. Bakt. (etc.) II Abt.*, 32:97-137.



# THE VARIATION OF HYDROGEN ION CONCENTRATION WITH CARBON DIOXIDE PRESSURE ABOVE ONE ATMOSPHERE

## I. COLORIMETRIC MEASUREMENT\*

MARJORIE B. MOORE WITH J. H. BUCHANAN

*From the Department of Chemistry, Iowa State College*

Accepted for publication March 11, 1930

## INTRODUCTION

It is known that the hydrogen ion concentration of neutral water changes very rapidly with small carbon dioxide pressures, and that this concentration continues to increase up to a pressure of one atmosphere, where the solution becomes 0.0338 molar in  $\text{CO}_2$  at  $25^\circ$  (6). Kendall (1) found the first ionization constant at  $25^\circ$  to be  $3.50 \times 10^{-7}$  for the concentration range corresponding to less than 1 atm.  $\text{CO}_2$  pressure. This constant is based upon the total  $\text{CO}_2$  in the solution, not upon the hydrated form only.

With the aid of the above data, one may readily calculate the hydrogen ion concentration expected for a  $\text{CO}_2$  pressure of 1 atm. at  $25^\circ$  to be  $1.088 \times 10^{-4}$  or  $10^{-3.96}$ .

It should be of interest to determine whether or not this increase of acidity continues as the pressure becomes much greater. In view of the known value of carbon dioxide as a germicidal agent, especially in the case of beverages, it should be a matter of both theoretical and practical importance to determine whether the germicidal action of carbon dioxide under moderate pressures may be due to the development of sufficiently high acidity to inhibit the growth of organisms.

## HISTORICAL AND THEORETICAL

Pfeiffer (2) and Haehnel (3) have measured the conductivities of  $\text{CO}_2$  solutions at pressures up to 25 atm. and 52 atm., respectively. Pfeiffer's experiments were carried out at  $0^\circ$  and at  $12.5^\circ$ , while the results of Haehnel are for  $0^\circ$  and  $15^\circ$ .

The hydrogen ion concentrations and ionization constants corresponding to the conductivity values found by these two authors have been calculated, and these calculated values, together with the original data, are tabulated in table 1. Pfeiffer's original paper gives conductivities in Siemens' units, but these have been converted into reciprocal ohms in this table.

The values for  $\Lambda^\infty$  at  $0^\circ$ ,  $12.5^\circ$ , and  $25^\circ$  were taken from the paper by Kendall (1). That for  $\Lambda^\infty$  at  $15^\circ$  was calculated from the value at  $18^\circ$  according to the data and empirical equation given by Kolthoff (4).

---

\*A thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.



Pressures used by Pfeiffer are not given in table 1, as his work was carried out at temperatures only approximately constant, and corrections introduced to bring the conductivity values to those for the exact temperature sought.

In the values of  $k_1$  there appears to be a break between 1 atm. and 5 atm. at 0°, according to the data of both the previously mentioned authors. Therefore we have averaged the values obtained above 1 atm. in each case. (Table 2.)

It will be seen that the agreement is good for 0°, but the values for 12.5° and 15° appear to be in the wrong order.

Moreover, it seems inconsistent that there should be such a decided drop of the ionization constant between one and five atm., with no appreciable further change as the pressure is increased.

A calculation of the ionization constant based on the hydrated portion of the  $\text{CO}_2$  was attempted. For this purpose, the straight-line function given by Shipley and McHaffie (5) was extrapolated. Their curve was obtained by plotting log of total concentration of  $\text{CO}_2$  (log  $m$ ) against log of fraction of the  $\text{CO}_2$  existing in the hydrated form (log  $n$ ). By extrapolation of their straight-line curve, values for  $n$  were obtained corresponding to the values for  $m$  used by Haehnel. Thus one may calculate values for the ionization constant,

$$K_1^1 = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$$

For a pressure of 5 atm., when  $m = 0.205$  at 15° one finds  $n = 8.14 \times 10^{-8}$ ; and the calculation for  $k_1^1$  gives it a negative value. This calculation is only approximate, as  $n$  was obtained for 18°, but it approaches exactness closely enough to show that to extrapolate such a function to a molar concentration as high as 0.205 is not legitimate. In fact, it seems to cast doubt upon the reliability of this function for even a limited concen-

TABLE 1. *Calculation of H-ion Concentration and ionization constants from conductivity measurements*

1. Haehnel.

(a) Temperature 0°

$$\Lambda_{\infty} = \Delta\text{H}^+ + \Delta\text{HCO}_3^- = 264$$

Pres- sure (atm.)	Vol. $\text{CO}_2$ per 1 Vol. $\text{H}_2\text{O}$ (0° 760mm.)	Mol. $\text{CO}_2$ per L. $\text{H}_2\text{O}$	Conductivity		Fraction ionized $\times 10^4$	H $\times 10_4$	pH	$K_1$ $\times 10^7$
			Specific L $\times 10_4$	Molar				
1	1.80	0.0804	0.140	0.174	6.59	0.530	4.28	3.49
5	8.71	0.389	0.667	0.171	6.48	2.52	3.60	1.63
10	15.89	0.709	0.919	0.130	4.92	3.49	3.46	1.72
15	21.82	0.974	1.063	0.109	4.13	4.02	3.40	1.66
20	26.53	1.184	1.186	0.100	3.70	4.48	3.35	1.62
25	30.46	1.360	1.250	0.0919	3.48	4.73	3.32	1.65
30	33.65	1.502	1.282	0.0854	3.23	4.86	3.31	1.57
35	36.73	1.640	1.318	0.0804	3.05	4.99	3.30	1.53
38	37.87	1.691	1.326	0.0784	2.97	5.02	3.30	1.49

(b) Temperature 15°

 $\Lambda_{\infty} = 340$ 

1	1.00	0.0446	0.4055	0.909	26.7	1.19	3.92	3.18
5	4.59	0.205	0.789	0.385	11.3	2.32	3.63	2.62
10	8.39	0.375	1.088	0.290	8.53	3.20	3.49	2.73
15	11.85	0.529	1.339	0.253	7.44	3.94	3.40	2.93
20	15.21	0.679	1.515	0.223	6.56	4.45	3.35	2.92
25	17.64	0.7875	1.678	0.213	6.26	4.93	3.31	3.09
30	20.31	0.907	1.764	0.194	5.71	5.18	3.29	2.96
35	22.52	1.005	1.831	0.182	5.35	5.38	3.27	2.94
40	24.44	1.091	1.911	0.175	5.15	5.62	3.25	2.89
45	25.59	1.142	1.940	0.170	5.00	5.71	3.24	2.855
50	27.06	1.208	1.971	0.163	4.80	5.80	3.24	2.78
52	27.67	1.235	1.983	0.161	4.73	5.85	3.23	2.77

Table 1 cont'd.

2. Pfeiffer.

(a) Temperature 0°

 $\Lambda_{\infty} = 264$ 

Vol. CO <sub>2</sub> per 1 vol. H <sub>2</sub> O (0° 760mm.)	Mol. CO <sub>2</sub> per 1 L. H <sub>2</sub> O M	Conductivity		Fraction ionized $\alpha \times 10^4$	$[H^+]$ $\times 10^4$	pH	$k_1$ $\times 10^7$
		Specific $L \times 10^4$	Molar $\Lambda$				
0.92	0.0411	0.277	0.673	25.5	1.05	3.98	2.67
0.95	0.0424	0.275	0.644	24.4	1.03	3.98	2.52
1.00	0.0446	0.296	0.664	25.15	1.12	3.95	2.82
1.67	0.07455	0.345	0.463	17.5	1.30	3.88	2.28
1.67	0.07455	0.341	0.458	17.3	1.29	3.89	2.23
1.68	0.0750	0.344	0.459	17.4	1.305	3.88	2.27
5.10	0.228	0.537	0.236	8.94	2.04	3.69	1.82
5.82	0.260	0.556	0.214	8.106	2.11	3.68	1.71
7.30	0.326	0.614	0.188	7.12	2.32	3.63	1.65
8.17	0.365	0.635	0.174	6.59	2.41	3.62	1.59
9.46	0.422	0.705	0.167	6.33	2.67	3.57	1.69
10.55	0.471	0.788	0.156	5.93	2.79	3.55	1.66
12.55	0.560	0.789	0.141	5.34	2.99	3.52	1.60
12.85	0.574	0.798	0.139	5.26	3.02	3.52	1.59
13.44	0.600	0.799	0.133	5.04	3.02	3.52	1.52
14.09	0.629	0.853	0.135	5.13	3.23	3.49	1.66
14.76	0.659	0.856	0.130	4.92	3.24	3.49	1.59
15.93	0.711	0.942	0.132	5.00	3.55	3.45	1.78
15.98	0.713	0.918	0.128	4.87	3.45	3.46	1.69
16.53	0.738	0.970	0.131	4.98	3.67	3.43	1.83
17.95	0.801	0.986	0.123	4.66	3.73	3.43	1.74
18.29	0.816	0.986	0.121	4.58	3.74	3.43	1.71
19.87	0.887	1.073	0.121	4.58	4.06	3.39	1.86
19.95	0.891	1.018	0.114	4.32	3.85	3.41	1.66
23.34	1.042	1.138	0.109	4.13	4.30	3.37	1.78

Table 1 cont'd.

(b) Temperature 12.5°

 $\Delta\infty = 323$ 

Vol. CO <sub>2</sub> per vol. H <sub>2</sub> O 0° 760mm.	Mol. CO <sub>2</sub> per 1 L. H <sub>2</sub> O	Conductivity		Fraction ionized $\alpha \times 10^4$ $\alpha \times 10^4$	$[H^+]$ $\times 10^4$	pH	$k_1$ $\times 10^7$
		Specific $K \times 10^4$	Molar $\Lambda$				
0.92	0.0411	0.390	0.949	29.4	1.21	3.92	3.55
0.95	0.0424	0.400	0.943	29.2	1.24	3.91	3.62
1.00	0.0446	0.421	0.944	29.2	1.30	3.89	3.80
1.67	0.0745	0.500	0.671	20.77	1.55	3.81	3.22
1.67	0.0745	0.491	0.658	20.4	1.52	3.82	3.10
1.68	0.0750	0.495	0.660	20.4	1.53	3.82	3.12
3.40	0.152	0.684	0.450	13.9	2.11	3.68	2.94
4.15	0.185	0.778	0.421	13.0	2.40	3.62	3.13
4.34	0.194	0.821	0.423	13.1	2.54	3.60	3.33
6.35	0.283	0.989	0.350	10.8	3.06	3.51	3.32
6.73	0.300	1.024	0.341	10.5	3.17	3.50	3.17
7.33	0.327	1.041	0.318	9.84	3.22	3.49	3.17
7.64	0.341	1.101	0.323	10.0	3.41	3.47	3.41
8.44	0.377	1.090	0.289	8.95	3.37	3.47	3.02
9.09	0.406	1.166	0.287	8.88	3.61	3.44	3.20
9.79	0.437	1.229	0.281	8.70	3.80	3.42	3.31
10.38	0.463	1.226	0.265	8.20	3.80	3.42	3.11
10.46	0.467	1.293	0.277	8.58	4.01	3.40	3.44
13.36	0.596	1.379	0.231	7.15	4.26	3.37	3.05
13.89	0.620	1.430	0.231	7.15	4.43	3.35	3.16
14.25	0.636	1.453	0.228	7.06	4.49	3.35	3.17
15.06	0.672	1.473	0.219	6.78	4.56	3.34	3.09
15.17	0.677	1.456	0.215	6.65	4.51	3.35	3.00
15.70	0.701	1.467	0.209	6.47	4.53	3.34	2.93
15.72	0.702	1.483	0.211	6.53	4.58	3.34	2.99
17.75	0.792	1.542	0.194	6.02	4.77	3.32	2.87
19.45	0.868	1.644	0.189	5.85	5.08	3.29	2.97
20.03	0.894	1.665	0.186	5.74	5.13	3.29	2.35

tration range, which is not dispelled by a careful consideration of the method used in obtaining it. Shipley and McHaffie's data are calculated from theoretical consideration of the results obtained in titration of solutions of carbonates and rest on the assumed constancy of the product  $[H^+][CO_3^-]$ . They found this product to remain practically constant in alkaline solutions, and assume the constancy to hold also in acid solutions. In deriving values for  $n$  they use Walker and Cormack's value (7) of  $3.0 \times 10^{-7}$  for  $k_1$  and assume that their theoretical values of  $k_1^1$  bar the relation to the value of  $k_1$ :

TABLE 2. Ionization constants of carbonic acid at high pressures

Temperature	$k_1 \times 10^7$	
	Haehnel	Pfeiffer
0°	1.61	1.69
12.5°	—	3.13
15°	2.86	—

$$k_1^1 = \frac{k_1}{n}, \text{ or } k_1^1 = \frac{3.04 \times 10^{-7}}{n}.$$

However,  $n$ , according to their calculations, appears to be of nearly the same order of magnitude as  $a$ , and hence  $a$  cannot be neglected legitimately in the denominator of the exact expression for the relation between these two forms of the ionization constant:

$$k_1^1 = k_1 \frac{1 - a}{n - a}.$$

As no other authors have attempted to express the fraction of  $\text{CO}_2$  hydrated as a function of concentration, a calculation of  $k_1^1$  cannot be made for pressures above 1 atm.

#### EXPERIMENTAL

In order to determine colorimetrically the pH in solutions of carbon dioxide at pressures above one atmosphere, it was necessary to design an apparatus whereby the gas could be admitted under various pressures and allowed to reach equilibrium with its solution before readings were taken.

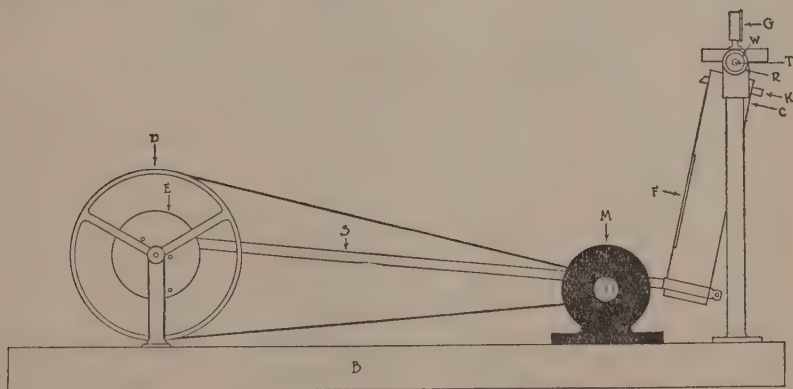


Fig. 1.

The final form of apparatus used is sketched in the accompanying diagram (Fig. 1). The arrangement of the complete apparatus is there shown. Fig. 2 shows a front view of the apparatus, and gives more details of the parts which are peculiarly adapted to this work. On opposite sides, the brass-case (C) contains slits (F), thru which the liquid in the bottle (P) may be viewed. A rubber gasket is interposed between the bottle and the brass head holding the gauge (G). The head may be firmly clamped onto the top of the bottle by means of the key (K) which is driven through slits in the case. Gas from the supply tank may be admitted thru the



flexible copper tubing (T) which extends through the wooden shoulders (W) swinging in the supports (R).

In order to make a series of colormetric determinations, 192 cc. of conductivity water and 8 cc. of 0.04 per cent solution of brom phenol blue are placed in a six and one-half ounce bottle, and  $\text{CO}_2$  gas is bubbled in for an hour under the atmospheric pressure in order to displace air dissolved in the water or present in the top of the bottle. The pH of the solution at 1 atm.  $\text{CO}_2$  pressure can now be read by comparison with the standards. This reading was found not to vary within the limit of accuracy of the experiment (0.1 pH unit) with any variation in barometric pressure in this vicinity.

After saturation of the water containing indicator with  $\text{CO}_2$ , the bubbling of gas is continued to prevent entrance of air while the bottle is placed in the apparatus and the key driven in. If the bubbling is slow, there will be only a small pressure of  $\text{CO}_2$  built up by this time. The needle-valve from the tank may now be closed and the apparatus shaken, shaking being continued for some time after there appears to be no further change in the indicator or in the gauge reading.

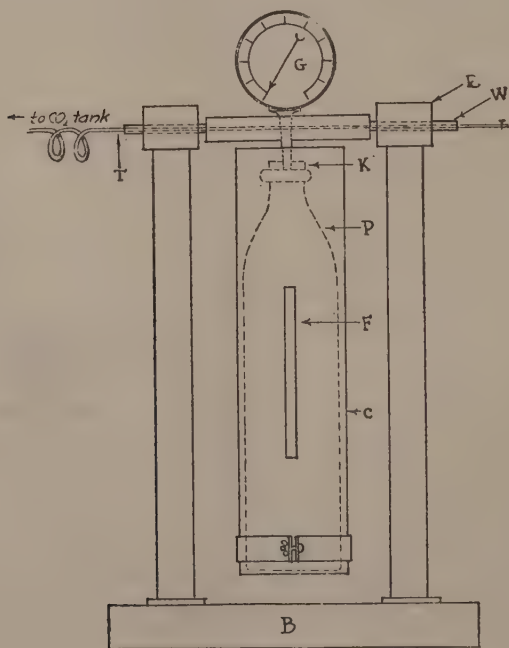


Fig. 2.

A series of readings may be taken on the same sample by increasing the pressure. The sample is again shaken until equilibrium is reached before its color is compared with the standards.

In order to maintain the temperature practically constant, the bottle of water was immersed for a considerable time either in a 25° thermostat ( $\pm 0.1^\circ$ ) or in a crock of melting ice which was frequently stirred. Water from the thermostat was used to maintain the temperature during the time the CO<sub>2</sub> was bubbled in and after the sample was clamped in the apparatus. After shaking to insure equilibrium, sufficient time was allowed for this temperature to be regained. These precautions appeared to be unnecessary, as it was not possible to read any change in the indicator corresponding to a considerable change in temperature, such as variations in the temperature of the room. Indeed, there is little variation in the results for 0° and 25°. It must be borne in mind, however, that the method is sensitive to 0.1 pH only.

The range of the indicator used was checked by the potentiometric method using a bubbling hydrogen electrode, and was found to extend from 3.0 to 4.6, which agrees with values previously reported for this indicator.

The "double standard" method was used for comparison. A total of 8 cc. indicator was used in each pair of standards, one bottle being sufficiently acid to insure all its indicator being in the acid form and the other alkaline so that this portion of the indicator was all in the alkaline form. The standards used were made up according to the scheme given in table 3, using a total volume of 200 cc. in each bottle, the bottles of course being similar to the one containing the sample.

TABLE 3. *Standards for colometric measurements of pH*

Pair	Bottle 1 (alkaline)	Bottle 2 (acid)	pH
1	0.5	7.5	3.1
2	1.0	7.0	3.2
3	1.5	6.5	3.3
4	2.0	6.0	3.4
5	2.5	5.5	3.5
6	3.0	5.0	3.6
7	3.5	4.5	3.7
8	4.0	4.0	3.8
9	4.5	3.5	3.9
10	5.0	3.0	4.0
11	5.5	2.5	4.1
12	6.0	2.0	4.2
13	6.5	1.5	4.3
14	7.0	1.0	4.4
15	7.5	0.5	4.5

For comparison, the sample to be measured is viewed through the vertical slit of the apparatus after inserting a bottle of distilled water in the line of vision to correct for any color present in the glass of the bottles. This color is then compared with a pair of standards, one of which is placed in a can containing vertical slits similar to the ones in the brass case of the apparatus. The pH of the solution is then taken as corresponding to the pair most closely matched. In this way it was found possible

to distinguish between the different pairs of the series, and hence to read the pH to 0.1 unit.

The bottles used were ordinary six and one-half ounce bottles such as are used for soda-water, and were quite uniform in color and thickness. The ones used in this work were selected on general appearance for uniformity. It was found that some of these bottles would withstand as much as 550 lbs. per sq. in. gauge pressure, or about 37 atm. None of the bottles used burst with pressure under 27 atm.

The CO<sub>2</sub> used was from a commercial tank. It had been analyzed by Mr. J. H. Toulouse and found to contain 99.7 per cent CO<sub>2</sub>, 0.050 per cent O<sub>2</sub> and 0.003 per cent CO. Hydrocarbons and SO<sub>2</sub> were found to be absent.

The results obtained by the colorimetric method are tabulated in table 4. In cases where a good matching of colors was not obtained, the second decimal figure in the pH reading was roughly estimated, these figures being added in parenthesis in the table.

TABLE 4. *pH of CO<sub>2</sub> solutions as determined colorimetrically*

Temperature 25°		Temperature 0°	
CO <sub>2</sub> Pressure		CO <sub>2</sub> Pressure	
( atm.)	pH	(atm.)	pH
1.0	3.7	1.0	3.5
1.7	3.5(3)	1.4	3.4
2.5	3.4(3)	2.6	3.3(2)
2.9	3.4(2)	3.6	3.3
3.7	3.4	8.3	3.3
3.8	3.4	15.3	3.2(7)
5.4	3.3(3)	23.4	3.2(5)
5.8	3.3		
7.2	3.3(2)		
7.8	3.3		
9.5	3.3		
10.5	3.3		
12.7	3.3		
18.7	3.3		
33.3	3.3		

Above 9.5 atmospheres, at 25°, there appeared to be very little change in the color of the sample as the CO<sub>2</sub> pressure was increased. At the highest pressures used, it appeared to match the pair corresponding to pH 3.3 better than that corresponding to pH 3.2, although the more nearly even matching of colors with the former pair was obtained at about 9.5 atm.

This apparent tendency to approach a limiting pH value must not be due to an indicator error at these high pressures, as other indicators failed to give any change corresponding to pH of 3.0 or 2.8.

This limiting value is not decreased by addition of citric acid to give an initial value of 3.5. Neither is there any decrease in pH of a citric acid solution initially at pH 2.2 with CO<sub>2</sub> pressure up to 26.5 atm.

The colorimetric results obtained are probably accurate to 0.1 pH unit at the pressure considerably above 1 atm. At low pressure the error may be somewhat greater, due to the fact that [H<sup>+</sup>] is so small that the ionization of the indicator itself may be not inappreciable in comparison.

However, ionization constants calculated from the colorimetric pH determinations must be quite inaccurate, in comparison with those obtained by the conductivity method. This may be seen readily from consideration of the fact that an error of 0.1 in the pH reading at a pH of 3.3 would mean an error of approximately  $1 \times 10^{-4}$  in the  $[H^+]$ , or about 20 per cent error. Hence such calculations are not made from the colorimetric data given in the foregoing discussion.

#### SUMMARY AND CONCLUSIONS

1. Values for the first ionization constant of carbonic acid,

$$\frac{[H^+] [HCO_3^-]}{[H_2CO_3 + CO_2]} = k_1$$

have been calculated from the conductivity measurement of Pfeiffer and of Haehnel at high pressures of carbon dioxide.

2. Calculation of the ionization constant,

$$\frac{[H^+] [HCO_3^-]}{[H_2CO_3]} = k_1^1$$

has been attempted using extrapolated values taken from Shipley and McHaffie's curve for the fraction of the total  $CO_2$  existing in the hydrated form at a given concentration. Such a calculation gives a negative value for  $k_1^1$  at five atmospheres  $CO_2$  pressure.

3. By a colorimetric method, the hydrogen ion concentration has been measured in  $CO_2$  solutions at a series of pressures up to 33 atm. at  $25^\circ$ , and to 23 atm at  $0^\circ$ . The pH of the solution was found to approach a limiting value between 3.3 and 3.2.

This is probably no greater acidity than that due to the citric acid added in the majority of carbonated beverages. As citric acid has been found to have no effect on the lower limit of pH obtained in  $CO_2$  solutions under the highest pressures studied, the power of  $CO_2$  to inhibit growth of organisms must be due, at least in part, to some factor other than the increase of hydrogen ion concentration in the solution.



## LITERATURE CITED

- (1) KENDALL, J.  
1916. The specific conductivity of pure water in equilibrium with atmospheric carbon dioxide. *Jour. Amer. Chem. Soc.* **38**:1480-1497.
- (2) PFEIFFER, E.  
1884. Ueber die electrische Leitungsfähigkeit des kohlensauren Wassers und eine Methode, Flüssigkeitswiderstände unter hohen Drucken zu messen. *Ann. Phys. Chem.* **33**:625-650.
- (3) HAEHNEL, O.  
1920. Über die Stärke der bei höherem Druck hergestellten wässrigen Kohlensäure. *Centbl. Min. Geol.* 1920:25-32.
- (4) KOLTHOFF, I. M.  
1923. Konduktometrische Titrationen. p. 11. Theodore Steinkopff, Dresden und Leipzig.
- (5) SHIPLEY, J. W. AND I. R. McHAFFIE  
1923. The bicarbonate equilibrium. *Jour. Soc. Chem. Ind.* **42**:319-326T.
- (6) LEWIS, G. N. AND M. RANDALL  
1923. Thermodynamics and the free energy of chemical substances. p. 576. McGraw-Hill Book Co., New York.
- (7) WALKER, J. AND W. CORMACK  
1900. The dissociation constants of very weak acids. *Jour. Chem. Soc.* **77**:5-21.

# THE PROPERTIES OF STARCH WITH RELATION TO TIME OF FORMATION OF STARCH GELS\*

O. W. CHAPMAN WITH J. H. BUCHANAN

*From the Department of Chemistry, Iowa State College*

Accepted for publication March 11, 1930

## INTRODUCTION

When a suspension of starch in water is heated, the granules imbibe water and become swollen and distorted. Accompanying this imbibition there is a change in viscosity. When the heated mixture is allowed to cool a gel is formed, and upon standing a clear liquid separates leaving a more concentrated gel behind. This separation is known as syneresis, and occurs in many starchy foods, such as in canned sweet corn, where it is objectionable because of a demand for a product of uniform consistency. It is not unlikely also that syneresis of the starchy material may be in part the cause of the staling of bread.

The purpose of this work is to study the syneresis of starch gels, and because of the changes in viscosity which take place during their formation, to determine whether or not there is a relationship between the viscosity of the pastes and the subsequent behavior of the gels.

## LITERATURE REVIEW

Early studies on starch were confined almost entirely to microscopic investigation, including the determination of the so-called "gelatinization temperature". Results of these determinations are summarized by Reichert (23).

Ostwald (21) in 1913 suggested the possibility of employing viscosity measurements in the study of starches. He showed that the viscosity increases up to a temperature of 95°, after which there is a decrease, until at 120° the viscosity is less than at 90°. This decrease has been observed by other investigators also. Stocks (26) found that a freshly prepared potato starch suspension gave a higher viscosity reading than one that had been allowed to stand. Denham, Blair, and Watts (5) called this the "fall back", and attributed it to the syneresis of the paste, but they believed that in flour the gluten was responsible chiefly for the change. Harrison (12) believed the decrease to be due to a breaking down of the swollen grains, and found that the rate of heating affected the results. Harvey (13) noted a decrease in viscosity with time for potato and cassava starches, but failed to find a similar decrease for corn starch. Samec (24) found that a decrease in viscosity resulted when starch suspensions were heated at 120°, and concluded that this was due to a gradual hydrolysis of amylophosphoric acid. Tiebaekx (27) agrees that heating or long standing causes hydrolysis

---

\*This paper is a part of a thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

of amylopectin, which is accompanied by a decrease in viscosity. Zwicker (28) failed to find any such relationship. Dhar (6) believes that the decrease is connected with dehydration, while Mardles (18) attributes such changes to progressive gelation.

Various types of viscosimeters have been employed by different investigators. The Ostwald type has been found to be satisfactory by Denham, Blair, and Watts (5) provided that the instrument be designed to give a stream line flow, by Hatschek (14) provided that the rates of flow are the same in all cases, and by Farrow and Lowe (9) provided that the same instrument is used for all measurements. This type has been found unsatisfactory by others, including Mardles (18) and Harrison (12). Other types of viscosimeters employed include the Scott by McNider (17) and the Redwood by Ermen (8). Torsion viscometers have been widely used. Rask and Alsberg (22) used a Stormer. Buel (4) found that a Doolittle viscometer did not give satisfactory results. The Mac Michael instrument has been widely used in flour studies, as by Gortner and Sharp (11).

The principal objection to the measurements of starch suspensions by these methods is that they may be plastic rather than viscous. Bergquist (3) has pointed out that in order to measure viscosity of starch suspensions, the concentration must be small. Sharp (25) found flour suspensions to be plastic when the concentration is above nine per cent. Dunn (7) considered dough made from wheat flour to be essentially a plastic system. In their studies on starch pastes, Herschel and Bergquist (15) employed a Bingham plastometer as they found it to be more satisfactory indicator of consistency than viscosity measurements. Bergquist (3) found that the flow of starch paste is plastic at temperatures below 70°; at higher temperatures the flow is viscous. Many investigators, as Ostwald (21) have shown that viscosity measurements are sensitive to slight changes in the system, and so are of value in the study of the colloidal state, provided that the measurements are made under uniform conditions.

Various explanations for the syneresis of gels have been given, based upon the possible structures of the gels. Syneresis is defined by Holmes (16) as the separation of water solutions from highly hydrated substances, such as silicic acid gels. He observed the syneresis of such substances to be more pronounced when the gel is free to contract.

Arsem (1) believes that syneresis results because the structural arrangements of the units in a gel are inherently unstable, so that a further condensation of the associated phase is possible through the re-orientation of units, and the release of loosely combined molecules of the liquid phase. Barratt (2) associates syneresis with gels in which the fibrils are coarse. Fischer (10) states that when the solid phase of a gel is no longer adequate to enclose all the solution of colloid insolvent, the system tends to "sweat". The gel still exists but is no longer dry.

Stocks (26) observed that on standing starch gels separate into two phases, a more solid gel and a liquid phase, the latter containing very little starch. He attributes the phenomenon to the aggregation of the molecular complexes, the colloid becoming less dispersed.

In his studies on starch gels, Meyer (19) found that separation of the dispersing agent to be greater for gels of low concentration which had stood for some time. The separation was found to be hastened and increased

by gentle shaking of the tubes containing the gels. Since the separation appears to result from the pressure of the gel itself, Meyer believes that it is probable that the gel has a continuous net of structure. Gels of lower concentration show the greater separation, probably because they have a lighter and looser connecting net work.

Osborne (20) believes that the weaker gels hold part of the liquid phase by capillarity in the lattice of the solid phase, whereas in the stronger gels the water exists in solid solution in the substance of the frame work. This view is held because the weaker gels extrude more readily.

These views indicate that starch gels may be considered as consisting of two phases, in which the solid phase probably exists in some kind of an irregular fibril structure. Syneresis may be regarded as a separation of the free phase, and its cause the aggregation of the associated phase.

#### EXPERIMENTAL PART

##### VISCOSIMETRIC METHODS

Three types of viscosimeters were tested to determine their applicability to the determination of viscosity of starch pastes. An instrument of the Scott type was found unsatisfactory because when used at low temperature considerable amounts of starch settled out, and at higher temperatures the formation of clots prevented free flow, and because of the loss by formation, and because of the formation of surface films.

A Doolittle's Improved Torsion Viscosimeter proved more satisfactory, although some difficulties were encountered in its use. Chief among these were the formation of surface films, which clung to the rod suspending the cylinder so that, if not broken by continual stirring, there was a sheet of film rotating with the cylinder. It is probable that the agitation necessary to prevent film formation caused a variation in the results. Considerable loss resulted from evaporation, necessitating the frequent addition of water to keep the volume constant.

Viscosimeters of the Ostwald type were found to be more satisfactory than either of the others. In only a few instances was difficulty encountered due to clogging in the tube, and the particles could easily be kept in suspension by applying gentle suction to the tubes. Practically no loss by evaporation occurred, nor were surface films troublesome. Temperature was more readily controlled than with the other instruments, and determinations more quickly made. The chief objection was that only small concentrations could be used.

#### THE EFFECT OF CONCENTRATION AND TEMPERATURE UPON THE VISCOSITY OF STARCH SUSPENSIONS

Suspensions of corn, wheat, rice, and potato starches in water were prepared in varying concentrations and their viscosities determined at different temperatures. In each case there was a gradual increase in the viscosity up to a certain temperature, after which there was more rapid increase up to the point of maximum viscosity. The maximum value was followed by a decrease, or fall-back, in the readings. The changes were more pronounced in higher concentrations, the increases and decreases being scarcely perceptible in concentrations of less than two per cent when



the Ostwald viscosimeter was used. Even higher concentrations were required to show this effect when the Doolittle instrument was used.

In table 1 are shown readings made on the four starches with the Ostwald viscometer, when three grams of each starch were suspended in 100 c.c. of water. The values shown were obtained by dividing the time of flow for the suspension by the time of flow for water at the same temperature.

TABLE 1. *Viscosity of Starches with Increasing Temperatures*

Variety of starch	$\frac{t}{\text{to}}$ at a temperature of											
	60°	70°	80°	85°	87.5°	90°	92°	94°	95°	96°	98°	99.5°
Corn	2.5	2.9	2.9	4.1	4.4	4.7	6.3	10.9		15.1	16.2	15.9
Wheat	2.0	2.3	2.8	3.0	3.2	3.7	5.3		6.8		8.7*	8.6
Rice	1.9	2.3	2.3	3.1	3.1	3.7	5.3	9.0		10.2	10.98	11.0
Potato	2.0	3.7	11.9	21.0		25.4			14.4		6.9	3.5

\*Reading at 97°.

These results show that the maximum viscosity is reached at the lowest temperature by potato starch, then wheat, and corn, while rice requires the highest temperature. The order of the starches in decreasing average diameters is potato, wheat, corn, rice, thus indicating that the temperature of maximum viscosity depends in part upon the size of the granules.

#### THE DECREASE IN VISCOSITY OF STARCH SUSPENSIONS WITH CONTINUED HEATING

It may also be seen from the results recorded in table 1 that after the maximum viscosity has been reached, that there is, at least for corn, wheat, and potato starch, a decrease shown. This decrease was found to be more evident if the heating was continued, and also to be influenced by the rate of bringing the suspension to the desired temperature. This is shown by the results recorded in tables 2 and 3.

TABLE 2. *Decrease in Viscosity of Starches of the Same Concentration with Continued Heating*

Variety of starch	$\frac{t}{\text{to}}$ Time in minutes heating continued											
	0	5	10	15	30	45	60	75	90	105	120	
Corn	52.4	33.3	22.9	17.8	10.2	6.4	4.9	4.5	4.1	3.9	3.5	
Wheat	16.3	15.3	10.6	7.6	4.7	4.7	3.7	3.5	3.3	2.7	2.7	
Rice	10.2	6.1	4.1	3.7	3.1	2.7	2.7	2.5	2.5	2.5	2.5	
Potato	*	*	*	260	224	208	171	159	117	111	100†	

\*Too viscous to read.

†71 after 400 minutes.

The data in table 2 were obtained by suspending three grams of the starch in 100 c.c. of water, heating over a flame to the boiling point, then quickly transferring a measured volume of the suspension to the viscometer suspended in a boiling water bath. The first measurement in each case was made without delay. The data in table 3 were obtained in the same way, except that the suspensions were heated slowly to boiling. Measurements following the first were made at the intervals indicated in the tables.

TABLE 3. *Decrease in Viscosity of Starches of the Same Concentration with Continued Heating*

Variety of starch	$\frac{t}{\text{to}}$ Time in minutes heating continued										
	0	5	10	15	30	45	60	75	90	105	120
Corn	17.8	17.3	16.7	15.7	13.3	10.2	8.0	7.1	6.1	5.7	5.5
Wheat	7.1	7.8	7.5	8.0	8.0	8.0	7.8	8.1	7.8	7.5	7.5
Rice	9.8	10.6	11.0	11.6	10.4	10.2	9.6	9.0	8.6	8.4	7.6
Potato	121.	22.8	8.6	5.7	2.9	2.9	2.7	2.7	2.7	2.7	2.7

Note: Suspensions brought to temperatures in forty-four minutes.

The data in tables 2 and 3 show that a much higher viscosity reading is obtained when the suspensions are heated rapidly, and that the decrease, which occurs soon after the maximum is reached, is greater when the suspensions have been brought rapidly to temperature.

The relationship between the rate of decrease in viscosity to the average diameter of the granules is not marked, being readily evident only in the case of a considerable variation. When the heating was rapid, the order of the rate of decrease was potato, corn, wheat, rice; as compared to potato, corn, rice, wheat, and to the order of decreasing average diameter; potato, wheat, corn, rice.

#### THE EFFECT OF ADDED REAGENTS UPON THE VISCOSITY OF STARCH SUSPENSIONS

It is evident that the determination of the effect of added reagents must be attended with considerable difficulty because of the number of factors which affect such determinations, as the concentration, temperature, rate of heating, and mechanical treatment. However, some knowledge of the effect of added substances may be gained, provided that all determinations are made in the same manner.

The Doolittle viscosimeter was used for these determinations, because of the greater convenience in adding material after the determination of the starch in water alone. The additions could be made without appreciable change in starch concentration, because the evaporation necessitated the addition of water or some solution to keep the volume constant. This procedure was not found practical with the Ostwald, because of the use of smaller volumes, and because the loss by evaporation was small. Following this method, it was found that the addition of acids in concentrations as low as 0.0025 N. caused a decrease in viscosity which was much more pro-

nounced than for starch in water alone. The addition of alkalis caused a marked increase in the reading, followed by a decrease. Dilute salt solutions showed no appreciable effect upon the rate of decrease in viscosity, but when added in amounts sufficient to make the solution one-half molar with respect to the salt, it was found that sodium chloride had practically no effect, while sodium sulphate was found to retard the time required to reach maximum viscosity, and that tri-sodium phosphate caused a considerable increase in viscosity, and the attainment of the maximum value at much lower temperatures than when in water alone.

#### THE FORMATION AND SYNERESIS OF STARCH GELS

The study of gel formation and syneresis has involved the effect of concentration, rate of heating, amount of exposed surface, variety of starch, the time that the gels are allowed to stand, and the effect of certain added substances.

The experimental work was carried out by heating various concentrations of starch suspensions over a flame to the desired temperature, and transferring the resulting pastes to test tubes. Two sizes of tubes were used so that the effect of surface area might be determined. The larger tubes averaged 2.2 cm. in diameter by 18 cm. in length, while the smaller tubes varied from 1.2 to 1.5 cm. in diameter by 17 cm. in length. The smaller tubes were graduated so that the volumes could be read directly. The larger tubes were not graduated, so it was necessary to remove the contents for measurement.

Mold growth was prevented by the addition of small amounts of toluene, and by storing the tubes at a temperature of  $-2^{\circ}$  C. All tubes were stoppered to prevent loss by evaporation.

The tubes were used without any coating material, as preliminary trials showed that the starch gels did not cling to the walls of the tubes, so that treatment with vaseline or other material was unnecessary.

Preliminary experiments showed that neither the rate of heating nor the time of heating affected appreciably the rigidity of the gels or the amount of syneresis, so all of the suspensions were heated rapidly over a flame, and immediately transferred to the tubes. Since the temperatures to which the different varieties of starch must be heated in order that gels might be formed are so nearly the same, all of the mixtures were heated to the boiling point, thus assuring the formation of gels in each case.

In table 4 are shown the results of allowing starch gels to stand in the tubes for periods of fifteen and twenty days. The values given show that when the larger tubes were used, the amount of syneresis was greater, indicating that the amount of expressed liquid depends upon the amount of surface. That this is not due to the larger volume of gel is evident, as not only the actual volume of liquid is greater, but also the percentage. This value is based upon the total volume of material in the tubes.

The amount of syneresis is shown to be different for different varieties of starch. Corn starch is shown to exhibit somewhat the greatest amount of syneresis, then potato, wheat, and rice. Corn and potato starches gave very nearly the same values, while wheat and rice starches have much the same values, both being much less than for the first two. All of the starches express more liquid in dilute solutions than when more concentrated.

TABLE 4. *The Syneresis of Starch Gels*

Variety of starch	Grams of starch in 100 c.c.	Large test tubes				Small test tubes			
		Volume and percentage of separated liquid							
		After 15 days		After 20 days		After 15 days		After 20 days	
		c.c.	Pctg.	c.c.	Pctg.	c.c.	Pctg.	c.c.	Pct.
Corn	3	10.0	20.0	20.8	46.0	1.0	6.0	2.0	10.5
Corn	4	8.0	16.6	17.6	39.0	1.2	6.5	3.0	15.8
Corn	5			14.6	34.0	0.2	1.2	2.5	13.5
Corn	6	4.5	12.1	10.2	25.0	1.0	4.6	2.7	15.0
Wheat	3	11.0	25.0	8.3	18.4	0	0	0.2	1.0
Wheat	4	7.0	14.6	5.3	12.3	1.6	8.6	0.1	0.5
Wheat	5	10.0	21.7	3.6	8.4	1.5	8.2	0.7	4.0
Wheat	6	1.0	2.6	1.3	3.3	0	0	0.1	0.5
Rice	3	5.0	10.8	9.5	1.9	2.2	13.3	1.3	7.9
Rice	4	0	0	0	0	1.5	8.5	0.6	3.4
Rice	5	0	0	0	0	2.0	10.8	0	0
Rice	6	0	0	0	0	0	0	0	0
Potato	3	5.0	10.2	17.7	39.3	0	0	4.5	24.0
Potato	4			14.9	36.0			3.0	13.9
Potato	5	1.0	2.2	10.5	27.0	0	0	1.5	8.6
Potato	6	5.5	13.7	10.0	26.0	3.0	12.1	2.5	15.0

That syneresis is a progressive phenomenon is shown by a comparison of the amounts of liquid expressed by the gels in fifteen days with the amounts expressed in twenty days. In all the tubes in which any appreciable amount of syneresis was shown, some liquid was separated at the end of one day. The amount increased rapidly for a few days, when the rate of increase became slower, although it continued for several weeks.

The addition of dilute acids to the suspensions of starch in water prevented the formation of gels. The addition of small amounts of dilute alkalis to the pastes greatly reduced the rigidity of the gels formed. When large amounts were added, gels were not formed.

To study the effect of other added substances upon the syneresis of starch gels, a series of suspensions of corn starch was prepared by the addition of four grams of the starch to 100 c.c. of water, together with the weight of the salt used, such as required to make the salt concentration one-half molar. These suspensions were heated to boiling over a flame, transferred to large test tubes which were stoppered and stored at  $-2^{\circ}\text{C}$ .



Table 5 shows the effect of the presence of salts upon the syneresis of the gels. In addition to the salts listed,  $\text{AlCl}_3$ ,  $\text{FeCl}_3$ , and  $\text{SnCl}_4$  were used, but gels were not formed in the presence of these substances.

It appears that the acetate, sulphate, oxalate, and citrates have hastened the syneresis of the gels. The effect appears to be largely due to the anion, since sodium and ammonium sulphates produced nearly the same effect. All of the other salts appear to either prevent or retard syneresis, the effect being the most notable in the case of the salts of the alkaline earths, and of sodium iodide, tri-sodium phosphate, and potassium sulphocyanate.

TABLE 5. *The Syneresis of Corn Starch Gels in the Presence of Salts*

Salt added	15 days		15 days		30 days		60 days	
	cc.	Petg.	cc.	Petg.	cc.	Petg.	cc.	Petg.
NaCl	1.3	3.1	1.0	2.4	18.	36.	19.	46.3
$\text{NaNO}_3$	0	0	0	0	2.	4.4	16.	35.5
NaI	0	0	0	0	0	0	0	0
Na Acetate	15.5	36.9	20.5	37.2	21.5	44.8	23.	48.9
$\text{Na}_2\text{SO}_4$	14.5	40.8	17.	41.4	21.5	44.8	22.	46.8
Na Oxalate	12.5	29.	14.1	29.4	19.5	44.3	23	47.9
$\text{Na}_2\text{CO}_3$	0	0	0	0	0	0	0.5	1.2
NaK Tartrate	0	0			10	25.	20.	37.7
$\text{Na}_2\text{HPO}_4$	0	0	1.1	2.4	6.5	14.4	12.	29.0
$\text{Na}_3\text{PO}_4$	0	0	0	0	0	0	0	0
Na Citrate	20.	40.			23.	46.	25.	47.1
KCl	0.3	0.6	0	0	14.	26.9	15.	37.5
$\text{NH}_4\text{Cl}$	0.8	1.8	2.2	4.1	15.5	34.4	21	42.8
$\text{MgCl}_2$	0.2	0.4	0	0	12.	24.	18.	36.
$\text{CaCl}_2$	0	0	0	0	0	0	0	0
$\text{SrCl}_2$	0	0	0	0	0	0	0	0
$\text{BaCl}_2$	0	0	0	0	0	0	0	0
$\text{ZnCl}_2$	0	0	0	0	0	0	5	11.1
KCNS	0	0	0	0	0	0	0	0
$(\text{NH}_4)_2\text{SO}_4$	18.5	38.5	16.5	36.6	21.	42.8	21.	46.6
$\text{Ca}(\text{NO}_3)_2$	0	0	0	0	0	0	0	0
Controls	8	16.5	8.	16.	18.5	37.7	21.	42.8
					25.	38.4	22.	44.9
					17.5	37.2	21.	46.6
					15.	24.2	10	25.0

The experimental work has shown:

1. That the amount of syneresis increases as the surface area of the gel increases.
2. That it increases with the age of the gel.
3. That it decreases as the concentration increases, with the corresponding increase in viscosity.
4. That it is not greatly affected by rate of length of heating.
5. That it may be retarded or prevented by the addition of certain salts.
6. That there is no relationship between the average diameter of the granules and the amount of syneresis.

#### SUMMARY AND CONCLUSIONS

It has been shown that there is a fall-back in viscosity of starch pastes after the maximum value has been reached, and that the gels formed from the paste exhibit syneresis. Both of these effects are believed to be due to a rearrangement of gel aggregates which liberates some of the water enclosed by the original structure.

Starch pastes of low concentration do not form as firmly bound gel aggregates as those of higher concentrations, and so are less viscous, and since their movement is less hampered, there is a greater degree of syneresis.

Syneresis may be prevented or retarded by the addition of certain salts. This effect is believed to be due to the formation of firmer gel structures, which do not readily enter into rearrangements permitting the expression of water.

#### LITERATURE CITED

1. ARSEM, WM. C.  
1926. Gel structure. *J. Phys. Chem.*, **30**:306-311.
2. BARRATT, J. G.  
1920. Structure of gels. *Chem. Age*, **3**:473.
3. BERGQUIST, CARL  
1925. Plasticity of starch paste. *J. Phys. Chem.*, **29**:1264-5.
4. BUEL, H.  
1912. A study of some physical properties of starch. 8th Internat. Cong. of Applied Chem., **13**:63-76.
5. DENHAM, H. J., G. W. SCOTT BLAIR, AND G. WATTS  
1927. Notes on the use of Ostwald viscosimeters for flour suspensions. *Cereal Chemistry*, **4**:206-220.
6. DHAR, N. R.  
1925. Viscosity in colloids in the presence of electrolytes. *J. Phys. Chem.*, **29**:1556-1567.
7. DUNN, J. A.  
1926. Plasticity: Its possibilities in cereal research. *Cereal Chem.*, **3**:351-359.
8. ERMEN, W. F. A.  
1907. The examination of commercial starches. *J. Soc. Chem. Ind.*, **26**:501-504.
9. FARROW, F. D., AND G. M. LOWE  
1923. Flow of starch paste through capillary tubes. *J. Text. Inst.*, **14**:414-440 T.
10. FISCHER, MARTIN H.  
1921. Soaps and proteins. John Wiley and Sons, New York. pp. 74-76.

11. GORTNER, R. A., AND P. F. SHARP  
1923. The physico-chemical properties of strong and weak flours, III. Viscosity as a measure of hydration capacity and the relation of the hydrogen ion concentration to imbibition in the different acids. *J. Phys. Chem.*, **27**: 481-492.
12. HARRISON, W.  
1911. On some properties of starch relating to its stiffening power. *J. Soc. Dyers and Colourists*, **27**:84-88.
13. HARVEY, E. H.  
1924. Some physico-chemical properties of starch. II. *Am. J. Pharm.*, **96**: 816-822.
14. HATSCHKE, EMIL  
1913. Die Viskosität der Emulsoid-ole und ihre Abhängigkeit von der Schergeschwindigkeit. *Kolloid-Z.*, **13**:88-96.
15. HERSCHEL, W. H., AND CARL BERGQUIST  
1921. The consistency of starch and dextrin pastes. *Ind. Eng. Chem.*, **13**:703-706.
16. HOLMES, HARRY N.  
1923. Gel formation. Colloid symposium monograph (Univ. Wisconsin). pp. 24-37.
17. MACNIDER, G. M.  
1917. A practical method for determining viscosity of starch for mill purposes. *Ind. Eng. Chem.*, **9**:597-9.
18. MARDLES, E. W. J.  
1923. Reversible sol-to-gel transition in non-aqueous systems. I. Change of viscosity with time during gelation. II. Viscosity changes associated with the gel-to-sol transition. *Trans. Faraday Soc.*, **18**:327.
19. MEYER, A.  
1913-1914. Beiträge zur Kenntnis der Gallarten, besonders der Starke-gallarten. *Kolloid chem. Beihefte*, **5**:1-18.
20. OSBORNE, W. A.  
1918. The theory of gel structure. *Proc. Roy. Soc. Victoria*, **30**:153-158.
21. OSTWALD, W.  
1913. Über die Bedeutung der Viskosität für das Studium des Kolloiden Zustandes. *Kolloid-Z.*, **12**:213-222.
22. RASK, O. S., AND C. L. ALSBERG  
1924. A viscosimetric study of wheat starches. *Cereal Chem.*, **1**:7-26.
23. REICHERT, EDWARD T.  
1913. The differentiation and specificity of starches in relation to genera, species, etc. Carnegie Inst., Washington. Pub. Part I. 174-175.
24. SAMEC, M.  
1911-1916. Studien über Pflanzen kolloide. *Kolloid chem. Beihefte*, **3**:123-60. **4**:132-174. **6**:23-54. **8**:33-62.
25. SHARP, PAUL FRANCIS  
1926. Wheat and flour studies. V. Plasticity of simple flour in water suspensions. *Cereal Chem.* 340-55.
26. STOCKS, H. B.  
1917. Colloid chemistry of starch, gums, hemicelluloses, albumin, casein, gluten and gelatine. First Report on Colloid Chemistry and its General and Industrial Applications. London. pp. 46-78.
27. TIEBACK, F. W.  
1923. Iets over Zetmeel. *Pharm. Weekblad*, **60**:338-9.
28. ZWIKKER, J. J. LIJNST  
1921. Contribution à la connaissance de la Fécule. *Rec. trav. chim.*, **40**:605-615.

## THE BIOLOGICAL ESTIMATION OF GLUCOSE

### II. THE RELATION BETWEEN GLUCOSE CONCENTRATION AND THE pH CHANGE IN MEDIA

AMY LEVESCONTE WITH J. H. BUCHANAN AND MAX LEVINE

*From the Department of Chemistry, Iowa State College*

Accepted for publication April 18, 1930

The factors affecting the rate of change in the pH of media inoculated with the organism *Aerobacter levans* have been presented in a previous article (3). The conditions observed included the original pH value, the buffer content, and the air supply of the media during incubation, as well as the original concentration of glucose when it was used as the only source of energy.

The results of this study indicated three conditions in which the pH changes of a synthetic medium were directly related to the original glucose concentration, thereby suggesting methods that might be used for the quantitative determination of glucose and other simple sugars in dilute solution.

The simplest method is based on the fact that in lightly buffered media containing less than .01 per cent glucose the increase in acidity or decrease in pH value is directly proportional to the original glucose concentration. This method would have only a limited application, however, since only very low concentrations could be studied, and since most biological fluids are highly buffered.

A second method, applicable to higher concentrations of glucose, depended on incubating the media in the absence of air. The only change that took place under these conditions was a production of acid caused by the decomposition of the glucose. The final pH value of the media, which was usually attained after five days incubation, was in direct proportion to the concentration of the glucose present. Solutions containing glucose in concentrations varying between .08 per cent and .32 per cent were studied by this method.

The third and most promising relationship between glucose concentration of media and the pH changes when inoculated with *Aerobacter levans* is based on the secondary change or increase in pH caused by the decomposition of the acids formed from the glucose. Media containing low concentrations in glucose begin to increase in pH value before those containing more glucose; and at the time this secondary change is taking place, the pH values of the media are a function of the original glucose concentration. This relationship and the factors affecting it have been discussed in the previous article.

In developing the third method a synthetic medium was prepared containing 0.10 per cent KCl, 0.10 per cent  $(\text{NH}_4)_2\text{HPO}_4$  and some bromthymol blue. Glucose solution was added to 100 c.c. portions of this to give definite concentrations. Media varying between .01 per cent and .40 per cent in glucose concentration have been prepared and studied.



The 100 c.c. of media were placed in 50 c.c. Erlenmeyer flasks in 25 c.c. portions. Each was inoculated with one c.c. of bacterial suspension, prepared as described in the previous article, and placed in a constant temperature water bath at 30° C. When the change in color of the brom-thymol blue indicated that the secondary change or increase in pH was beginning, usually after about 24 hours, the pH value was determined electrometrically.

In applying this technique to the determination of glucose in an unknown solution, media could be prepared in a similar manner with control media of the same composition, both inoculated with the same bacterial suspension and incubated in the same way. By comparing the pH value attained by the unknown solution with those attained by the control media, the concentration in the unknown media could be interpolated.

This method has been applied in the determination of the glucose formed by inversion of sucrose in a slightly acid medium.

#### ACCURACY OF RESULTS

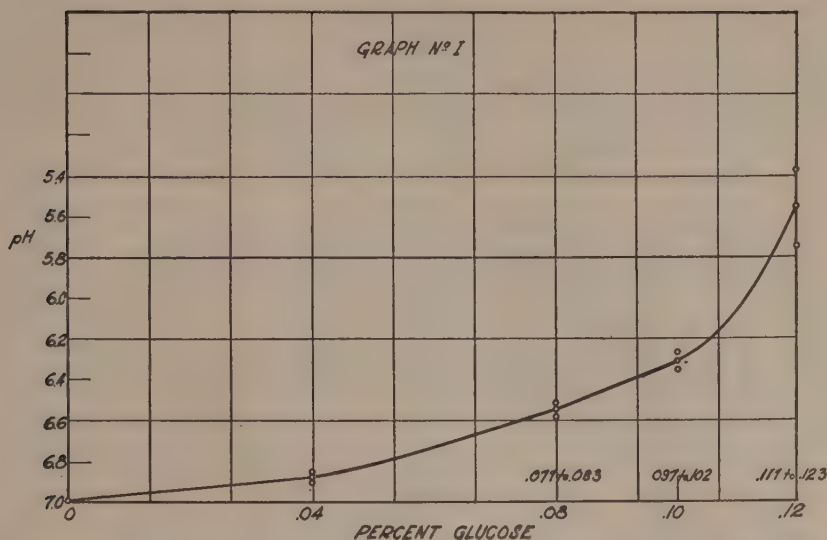
The accuracy of a biological method for quantitative analysis is dependent on the reliability of the organisms acting in a similar manner under the same circumstances. Altho in complex media there is less probability of the reaction of the bacteria being identical in two cases, it has been shown by Kendall (2) that bacteria use carbohydrates as a source of energy in preference to protein. This would mean that in the presence of protein and one available carbohydrate, the changes would be consistent since the rate of change would depend only on the activity of the organisms.

In the method outlined, the media at the beginning contain only one available carbohydrate. As soon as acids are formed, these become a possible source of energy if incubated under aerobic conditions, and then there is opportunity for variation in the action of organisms. Since the decomposition of acid tends to increase the pH value and the decomposition of sugar tends to decrease it, the error would be doubled if acid were being decomposed in one tube while sugar is used in another.

To determine the error that should be expected, several determinations were made of the change in pH of replicate media after 24 hours of incubation. Table 1 and Graph I show the pH value obtained. The probable error of each result is calculated as two-thirds of the standard deviation. This is marked on the graph, and by drawing a line from these points to the curve, the point is found that represents the concentration of glucose to which this error in pH corresponds.

TABLE 1. *The pH of replicate tubes of media containing varying concentrations of glucose*

Original glucose	pH of media after 24 hrs. incubation									Average	Pctg. glucose
.04	6.87	6.87	6.90	6.88	6.87	6.87	6.88	6.89	6.88	6.88±.01	.04±.001
.08	6.56	6.63	6.52	6.54	6.59	6.61	6.46	6.49	6.56	6.55±.03	.08±.002
.10	6.27	6.28	6.28	6.34	6.27	6.36	6.40	6.42	6.28	6.32±.04	.10±.002
.12	5.19	5.16	5.83	5.93	5.54	5.83	5.48	5.46	5.70	5.57±.18	.12±.025



Graph I. The pH of replicate tubes of media containing varying concentrations of glucose.

A variation of 0.01 pH at 0.04 per cent glucose concentration represents as great an error in the determination, as a variation of 0.2 pH at a point at which the curve is rising rapidly. This is counteracted because the variation in pH readings at 0.04 per cent concentration is very small. The probable error in all of the four concentrations studied is approximately 2.5 per cent.

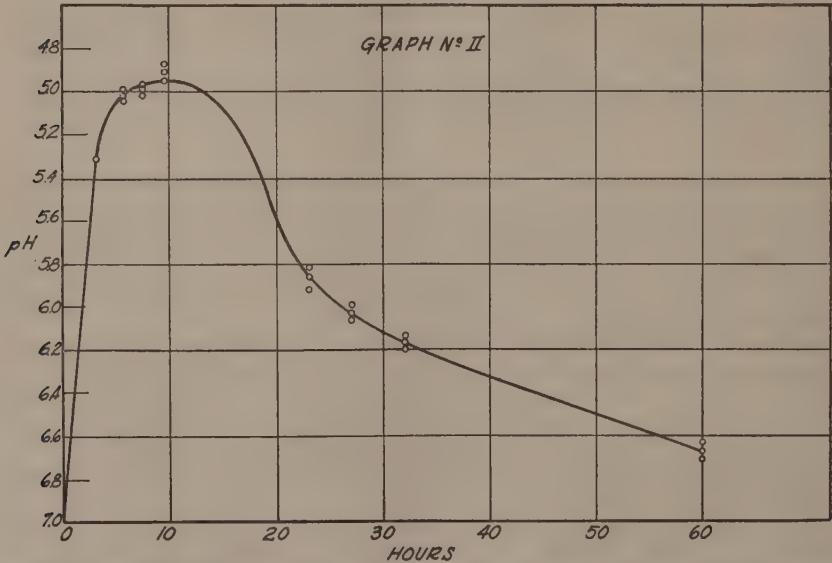
The time interval of 24 hours was chosen because previous experiments had shown that near this time there is the greatest range of pH in media containing varying concentrations of glucose. This time corresponds approximately to the time when all media are increasing in pH value, immediately after the minimum pH of the higher concentrations has been reached. It is at this time that both the glucose and the organic acids are available as sources of energy, and the opportunity for variation is greatest. To study this, a comparison was made of the variation in pH values of a medium containing 0.10 per cent glucose after different intervals of incubation. The results, given in table 2 and Graph II, indicate that the error does increase after the acids have become available as a source of energy.

The readings of pH after three hours showed no variation, since only glucose had been available. At five hours, when acids have begun to be used, the probable error is  $\pm 0.02$  and after seven hours the error is consistently about  $\pm 0.04$ . It would probably be advisable in an experiment to determine the pH values immediately after the pH of the highest concentrations of glucose begin to increase. At this time the variation in pH value due to original glucose concentration is greatest, and the probable error is no greater than at later times. In an experiment using media of different composition, it would be necessary to make a preliminary test to

determine the incubation time that would represent this stage of the re-action.

TABLE 2. *The pH of replicate tubes of media, after different periods of inoculation*

No. hours incubated	pH of media after incubation								Average
3	5.32	5.32	5.34	5.32	5.32	5.32			5.32±0
5½	5.06	4.97	5.02	5.00	5.05	4.98	5.04	5.04	5.02±.02
7½	4.97	5.00	4.93	4.99	5.04	5.06			5.00±.03
9½	4.88	4.92	5.02	4.88	4.86	4.95			4.92±.04
23	5.88	5.70	5.97	5.93	5.88	5.88			5.87±.05
27	6.13	6.12	6.02	6.00	6.02	5.97	6.02		6.04±.04
32	6.20	6.24	6.08	6.22	6.12	6.24	6.13	6.15	6.17±.04
60	6.72	6.67	6.77	6.63	6.70	6.59	6.65		6.68±.04



Graph II. The pH of replicate tubes of media after different periods of incubation.

#### DETERMINATION OF INVERSION CONSTANTS OF SUCROSE

Because of their selective action, biological reagents are especially applicable for the analysis of sugar mixtures. Since *Aerobacter levans* does not assimilate sucrose, this organism can be used to advantage for the determination of sugars present in minute quantities in a solution of sucrose in a higher concentration. This analysis is difficult to carry on with chemical reagents, but is necessary for the determination of the rate of inversion of sucrose in the presence of a weak acid.

A determination of invert sugar by the action of *Aerobacter levans* is possible if glucose and fructose are decomposed by the organism indis-

criminally. Slator (4) found that these sugars were fermented by yeasts at equal rates and Willstatter and Sobotka (5) observed that enzymes react with them at the same speed. A comparison was made of the rates of acid production from the two sugars by *Aerobacter levans* by inoculating similar media containing varying concentrations of chemically pure glucose and fructose. The results given in table 3 demonstrate that the rates are similar, and that the organism may be used to determine the concentration of the combined sugars.

In order to determine the inversion constant, a 5 per cent solution of sucrose in .0153 N  $\text{H}_3\text{PO}_4$  was placed in a thermostat at  $30^\circ \text{C}$ . At various intervals 10 c.c. portions of the solution were removed. These were neutralized immediately and 10 c.c. of 0.2 percent  $(\text{NH}_4)_2\text{HPO}_4$ , 10 c.c. of 1 per cent KCl, and 2 c.c. of bromthymol blue solution were added to each portion. Each of these solutions was made up to 100 c.c. and sterilized in 25 c.c. portions in Erlenmeyer flasks. The control media were made from the same formula except that varying quantities of glucose were used instead of the sucrose.

The results were checked by a similar experiment in which the rate of inversion was followed by the use of a polariscope. The results obtained are given in table 4 and Graph III.

The graph is plotted from the pH values of the media containing known quantities of glucose. The concentration of glucose and fructose in the media containing partially inverted sucrose is interpolated from this graph. The inversion constant calculated from these determinations is approximately .005.

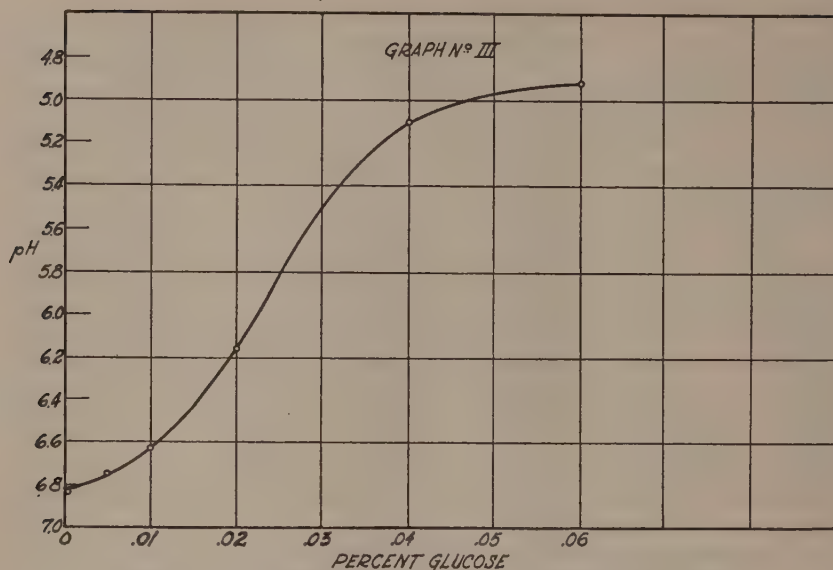
TABLE 3. Comparison of rates of pH change in media containing equal concentrations of glucose and fructose

pH change of glucose media			pH change of fructose media		
Conc. of glucose	Time		Conc. of fructose	Time	
	3½ hr.	7 hr.		3½ hr.	7 hr.
.04%	5.93	6.32	.04%	5.95	6.34
.08%	5.18	5.32	.08%	5.31	5.40
.12%	5.14	4.92	.12%	5.16	4.98
.16%	5.06	4.77	.16%	5.22	4.88

TABLE 4. Determination of inversion constant of sucrose

Control media		Inverted sucrose media				
Conc. of glucose	Average pH value	Time of inversion	Average pH value	Conc. of invert sugar	Inversion constant t=hours	Inversion constant from polariscope reading
.005%	6.75	6 hr.	6.25	.018%	.00610	.00729
.010%	6.63	10 hr.	5.88	.024%	.00491	.00894
.020%	6.17	26 hr.	4.96	.052%	.00422	.00796
.040%	5.11					
.060%	4.93					





Graph III. Determination of inversion constant of sucrose.

#### CONCLUSIONS

When the study of this problem began it seemed possible that a graph could be plotted from which readings could be made immediately of the concentration of glucose that corresponds to the pH developed in media after a definite period of incubation.

It will be seen from Graph III that it has been possible to plot the pH developed against the original concentration of glucose and to obtain a typical curve. It is possible also, under carefully controlled conditions, to duplicate these curves and to estimate the concentration of glucose in unknown media by the use of these curves.

Since the method is entirely empirical, and since there is no method known to determine the activity of an emulsion of bacteria, it would not be practical to depend upon a curve plotted from earlier experiments.

It is possible, however, to accomplish the same result in a more dependable method by inoculating control media of known glucose concentration at the same time and with the same emulsions of bacteria as the unknown media. The pH of all the media could then be determined at the same time, a graph drawn from the results with the control media, and the concentration of the unknown media interpolated. This is a method that has been applied in determining the inversion constant of sucrose. Preliminary experiments are often necessary to determine the optimum time to read the pH value, and to determine the optimum buffer value of the media. Usually the concentration of glucose in the unknown media is known well enough to make the control media of approximately the same concentration.

The use of control media, with the same emulsion of bacteria as the unknown media, not only avoids a serious error in this method but makes possible a change in the composition of media for any specific experiment. Frequently it is necessary to determine glucose in media more complicated

than that used in these experiments. By making the composition of the control media a duplicate of the unknown media, the method outlined here can be applied. In a complex biological fluid, it might be possible to remove the sugar to be determined from some of the liquid by a preliminary fermentation with the organism. The sugar could then be added to the resulting mixture in known quantities, and the pH change compared with the change in the fluid containing the unknown concentration of sugar. It would be necessary in such a determination to find an organism that reacted only with the sugar to be determined.

One of the most interesting applications of a biological method for determining sugars is in the determinations of changes in structure of the sugars under the influence of inorganic reagents. The rate of inversion of sucrose by phosphoric acid has been determined by this method. Another interesting application, suggested by Kendall (1), would be the determination of inter-conversion of glucose, mannose, and fructose in slightly basic solutions. An organism that is specific for glucose, and another specific for fructose and glucose, would be necessary for this determination. The fructose or mannose could be allowed to stand in a solution of NaOH for definite time intervals. The solutions would then be neutralized and nutrient salts added. With one organism the glucose concentration of the resulting solution would be determined, and, if the fructose were present in small enough concentration, it could be determined by subtracting the glucose concentration from the concentration of the combined sugars, determined by the second organism.

#### SUMMARY

1. The biological method of determining sugars is feasible under carefully controlled conditions.
2. A method has been developed for determining sugar in concentrations between .01 per cent and .40 per cent by the rate with which the secondary pH change takes place under aerobic conditions.
3. The accuracy of the method is from .001 to .025 per cent.
4. Glucose and fructose produce acid at the same rate under these conditions.
5. The inversion constant of sucrose in .015 N  $\text{H}_3\text{PO}_4$ , expressing "t" in hours, is about .005.

#### LITERATURE CITED

1. KENDALL, A. I.  
1921. Bacteria as chemical reagents. *J. Chem. Met. Eng.* 24:56-60.
2. KENDALL, A. I. AND C. J. FARMER  
1912. Studies in bacterial metabolism. I. *J. Biol. Chem.* 12:13-17.
3. LEVESCONTE, A., WITH J. H. BUCHANAN AND M. LEVINE  
1930. The biological estimation of glucose. I. A study of factors influencing changes in H ion concentration of media. *Iowa State College Jour. Sci.* 4:331-342.
4. SLATOR, A.  
1908. Studies in fermentation. II. Mechanics of alcoholic fermentation. *J. Chem. Soc.* 93:217-242.
5. WILLSTATTER, R. AND H. SOBOTKA  
1922. Ueber auswählende Garung von Zuckergemischen. *Z. physiol. Chem.* 123:170-175.



## DETERMINATION OF ORGANIC ACIDS

### II. DETERMINATION OF MIXTURES OF TWO FATTY ACIDS BY PARTITION BETWEEN ETHYL ETHER AND WATER

C. H. WERKMAN

*From the Bacteriology Section, Agricultural Experiment Station, Iowa State College*

Accepted for publication May 8, 1930

The method of E. Duclaux (2) is generally used by the zymologist for the routine quantitative determination of the fatty acids in dilute solutions. The method is based on the differences in the rates of distillation of the acids. Those who have used distillation methods have generally found them to be unreliable and time-consuming. Behrens (1) and Werkman (3) have proposed the use of the differential distribution of the acids between immiscible solvents as a more convenient and accurate approach. The former proposed the use of the distribution between ethyl ether and water, titrating the acid present in both phases and algebraically solving for the quantities of the acids by the use of simultaneous equations. Werkman pointed out the advantages of the system of isopropyl ether and water and standardized the procedure for routine work to provide for quantitative determination by reference to graphs. The purpose of the present paper is to extend the method of graphical determination and standardization to the ethyl ether-water partition. The data for the ethyl ether-water partition will be required as a basis, for the quantitative determination and provisional identification of a mixture of two or more fatty acids, in a future communication. There are two reasons why the use of the ethyl ether-water partition is discussed and standardized for graphical solution. First, this system has one advantage over that of isopropyl ether and water: it is slightly more sensitive, and secondly, it is proposed to use a combination of the two systems in an extension of the partition method to the provisional identification of fatty acids in mixtures. For a discussion of the advantages and details of the partition method the reader is referred to papers (1) and (3).

#### METHOD

The partition method of fatty acid determination finds application in the same type of investigation as the Duclaux. It should prove of considerable value to the zymologist making routine determinations of the acids produced by the action of microorganisms. It may be used to determine the volatile and non-volatile acids or their mixtures. As here developed, the practicability of the method is limited to the determination of the relative proportions of two acids in solution. Thus the method has in general the same limitations as the Duclaux. The use of either method requires chemical identification. The partition method provides much greater accuracy than that of distillation and a determination may be performed in much less time.

Let it be assumed that a quantitative determination is to be made of the volatile acids in a fermented medium. A convenient quantity of the



medium is adjusted to pH 3, by the addition of  $\text{H}_2\text{SO}_4$  to liberate the fatty acids, and distilled.  $\text{NaCl}$  may be added to raise the boiling point. Direct distillation is continued to a minimum of residue to drive over the volatile acids. The distillate is adjusted to a fixed normality such as 0.1 N. This will be determined by the type of investigation. To 30 cc. of the 0.1 N solution are added 20 cc. of ethyl ether in a separatory funnel and the whole vigorously shaken for one minute. Three minutes are now allowed for the separation of the ether and water phases. Twenty-five cc. of the aqueous phase are withdrawn and titrated with 0.1N alkali. The number of cc. of 0.1N alkali required to neutralize 25 cc. of the aqueous phase may be termed the *partition constant*.

TABLE 1. *Experimental values of partition constants for mixtures of two acids between ethyl ether and water. 25cc. aqueous phase at 25°C.*

Percent 0.1 N acid (first named)	Butyric- acetic	Butyric- propionic	Propionic- acetic	Lactic- acetic	Lactic- butyric	Percent 0.1 N acid (second named)
100	8.1	8.1	12.4	22.5	22.45	0
90	9.2	8.6	13.0	22.2	21.0	10
80	10.3	9.0	13.75	21.9	19.6	20
70	11.4	9.5	14.3	21.6	18.1	30
60	12.5	9.9	15.0	21.2	16.7	40
50	13.7	10.3	15.8	20.9	15.2	50
40	14.7	10.8	16.4	20.6	13.8	60
30	15.8	11.1	17.1	20.2	12.4	70
20	17.0	11.6	17.8	19.9	11.0	80
10	18.1	12.0	18.5	19.6	9.6	90
0	19.2	12.4	19.2	19.2	8.1	100

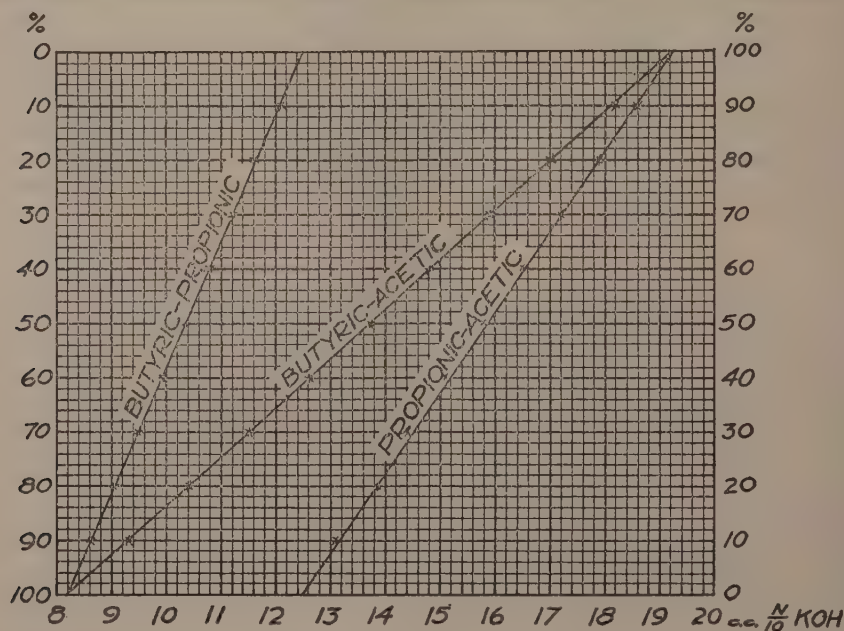


Fig. 1. Partition of a mixture of two acids between ethyl ether and water

The reading of the *partition constant* is located on the appropriate graph in figure 1 and the proportion of each acid determined by reference to the proper percentage column. Percentages on the left refer to the first named acid; those on the right refer to the second named acid. It is apparent from the graph of figure 1 that the plots of *partition constants* against relative percentages of acids are straight lines. There is, therefore, no difficulty in interpolating. In fact, the entire graph may be constructed by determining the terminal points for the particular sample of ether at the disposal of the investigator. The *partition constants* shown in table 1 were obtained with Mallinkrodt ether to which sodium had been added to remove any alcohol or water present. Results obtained by the use of the method are shown in table 2. It is to be observed that the error is well within that which is to be expected by the use of distillation methods.

TABLE 2. *Ratios of acids found in known mixtures of fatty acids*

Sample	Acids in solution*	Ratio %	Ratio found %
1	Propionic, acetic	60:40	60:40
2	Butyric, propionic	50:50	50:50
3	Butyric, acetic	30:70	31:69
4	Propionic, acetic	80:20	80:20
5	Butyric, lactic	50:50	49:51

\*Solutions in a mixture in distilled water.

Graphical solution offers a number of advantages over the algebraic method and is the method of choice for routine work; however, quantitative determination of two acids may be calculated from a knowledge of the partition coefficients of the acids present and the number of cc. of 0.1N KOH required to neutralize (a) total sample partitioned and (b) aqueous phase.

The general equations are as follows: it is assumed for purpose of illustration, that the acids are partitioned between equal volumes of ethyl ether and water; also, that the original solution is adjusted to 0.1 N.

$A_1$  = cc. 0.1 N first acid present in cc. of 0.1 N mixture.

$A_2$  = cc. of 0.1 N second acid present in x cc. of 0.1 mixture.

$P_1$  = partition coefficient of first acid.

$P_2$  = partition coefficient of second acid.

x = cc. 0.1 N alkali required to neutralize aliquot of original mixture  
= no. of cc. of original solution employed after adjustment to  
0.1 N 30 cc. have been used in the graphical solution.

w = cc. 0.1 N alkali required to neutralize aqueous phase when partition is made between equal volumes of ethyl ether and water.

$$A_1 + A_2 = x \quad (1)$$

$$\frac{1}{1 + P_1} A_1 + \frac{1}{1 + P_2} A_2 = w \quad (2)$$

$$\left( \frac{1}{1 + P_2} - \frac{1}{1 + P_1} \right) A_1 = \left( \frac{1}{1 + P_2} x \right) - w \quad (3)$$

$$A_2 = x - A_1 \quad (4)$$

The partition coefficient is defined as the ratio of distributions of acid between equal volumes of ether and water, the latter phase being unity, i.e. 0.434:1 for acetic; 1.73:1 for propionic and 6.28:1 for butyric.

It is not necessary to adjust the original solution to known normality. Determinations may be made by titrating both ether and aqueous phases and substituting for equation 1 an equation obtained similarly to equation 2 but applicable to the ether phase:

$$\frac{P_1}{1 + P_1} A + \frac{P_2}{1 + P_2} = U \quad (5)$$

U = cc. 0.1 N alkali required to neutralize ethyl ether phase.

The partition method as here described will not determine the proportions of three acids. It will give the limits of these proportions, which will serve to give a general idea as to the relative composition of the mixture. (See nomogram, Fig. 2).

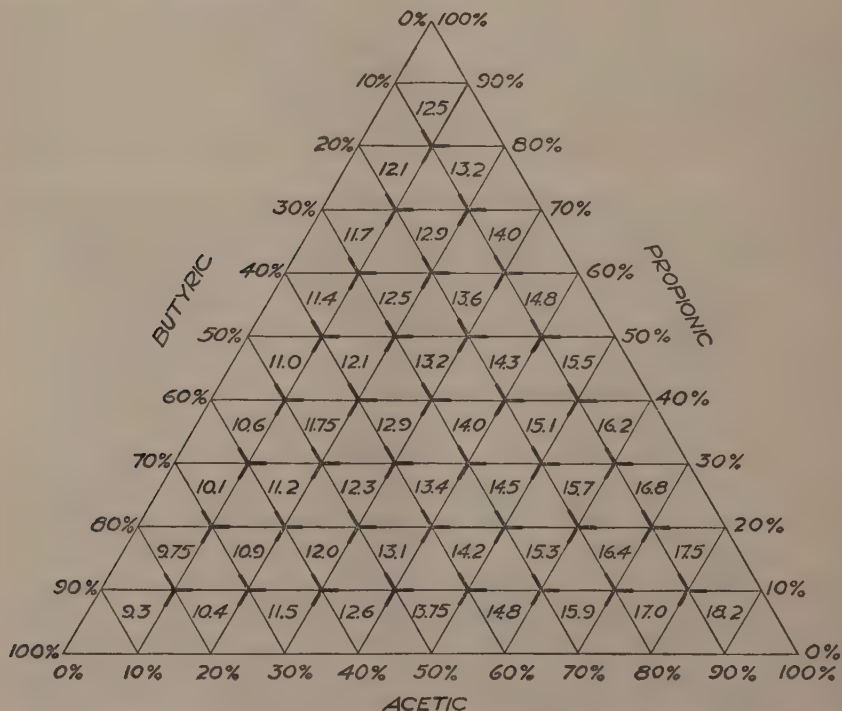


Fig. 2. Determination of organic acids in a mixture. Partition between ethyl ether and water

## DISCUSSION

The partition method as here developed is based upon the practicable utilization of the distribution of the fatty acids between ethyl ether and water in relatively dilute concentration. The graphs have been constructed from experimental data, therefore the question of the effect of solution phenomena requires no discussion. Consideration of the equations previously given, wherein use is made of the partition coefficient, might require attention to point out that it is not necessary to assume rigid constancy of the coefficient with changing concentration of acid in order to solve by the use of these equations. Undoubtedly when we are dealing with substances, such as the fatty acids which both associate and dissociate, the partition coefficient will not be constant over large changes of concentration. It is for this reason that a fixed normality is employed.

Concentration as a factor influencing the results, no longer requires discussion; the only effect to be considered is that of changing proportions of the acids which make up the total acidity. A 0.1 N mixture of propionic and acetic acids, for instance, may be composed of 99:1 or 1:99 parts respectively. That the effect of changing the proportions of the acids does not materially alter the partition coefficient of the mixtures is shown by experimental results. Such an alteration is not to be expected from the similarity in the dissociation properties of the two acids.

Study of the distribution of acids between immiscible liquid-liquid phases shows that in a majority of the cases mathematical consideration of dissociation and association is necessary to obtain constancy of the partition coefficient. On the other hand, there is no great change in the coefficient over a limited change of concentration and it is true that there are a number of substances in which the uncorrected ratio as determined by analysis is reasonably constant over a considerable range of concentrations. In view of this relative constancy with changing concentration, there is little change to be expected due to dissociation or association when the properties of the two acids are very similar.

The concentration of the solution of acids in the mixture has been kept low purposely in order to avoid the effect of any considerable concentration of solute on the mutual solubilities of the two liquid phases. Mutual solubilities must be kept constant to maintain constancy of the partition coefficient.

## SUMMARY

For the quantitative determination in mixtures of two acids in solution, the partition method using the system ethyl ether-water makes practicable the utilization of the differential distribution of fatty acids between two immiscible solvents. The procedure is simple, yields results more accurate, and is less time-consuming than the Duclaux. Moreover, it may be used with volatile or non-volatile acids or their mixtures.

The partition method should prove of value to the zymologist required to make quantitative fatty acid determinations. In forth-coming papers the partition method will be extended to greater usefulness in the identification and quantitative determination of organic acids.



## REFERENCES

1. BEHRENS, W. U.  
1926. Quantitative Analyse von Gemischen flüchtiger Fettsäuren durch Verteilung zwischen Äthyläther und Wasser. *Z. Anal. Chem.* **69**:97-107.
2. DUCLAUX, E.  
1900. *Traité de Microbiologie*, Masson et Cie., Paris. **3**:384-95.
3. WERKMAN, C. H.  
1930. Determination of organic acids. I. A new method for the determination of fatty acids in mixtures by partition between isopropyl ether and water. *Ind. Eng. Chem. (analytical edition)* **2**, (No. 3).

# THE ESTERASE AND PROTEASE OF *PENICILLIUM ROQUEFORTI*

N. M. NAYLOR, L. WEISBRODT SMITH, AND HELEN JO COLLINS<sup>1</sup>

*From the Chemistry Laboratory, Iowa State College*

Accepted for publication March 6, 1930

Experiments have shown the presence of proteolytic and lipolytic enzymes in a ripened Roquefort cheese and in milk on which the mold *Penicillium roqueforti* has been grown. The action of proteolytic enzymes upon casein and of lipolytic enzymes upon the fats of milk form certain volatile acids which Currie (2) considers may account for the characteristic taste of the Roquefort type of cheese. Hence it was thought that the conditions most favorable for the production of a good quality Roquefort cheese and the formation and activity of the proteolytic and lipolytic enzymes formed by growth of the mold were the same. Therefore, since milk or cheese is a complicated substance in which to study enzyme action a synthetic medium was used on which to grow the mold. Dox (3), Golding (8), Currie (2) and Thom (11) used Czapek's solution as a medium for the growth of *P. roqueforti* for cultural and enzyme studies. As these experiments provide considerable data for comparative purposes, it was decided to use Czapek's medium as a basis for these enzyme studies. In the literature, there is occasional reference to the stimulative action of ammonium salts.

Satio (9) reported an increase in the production of diastase by mold when ammonium salts were added to a certain nutrient medium. Fulmer and co-workers (5,7) used ammonium salts in a medium in which they studied yeast growth. They found a definite relationship to exist between yeast growth and temperature, hydrogen ion, and concentration of ammonium salts of the yeast nutrient medium. In view of the experiments previously cited, it seemed possible that the presence of ammonium salts in Czapek's medium might favor mold growth.

The work here reported consisted of: (a) studies on the composition of a medium which will give good growth of mold and consequent production of esterase and protease; (b) digestion experiments in which the optimum conditions for the activity of the enzymes are studied.

## I STUDIES ON THE MEDIUM

These studies were planned to determine the concentration of ammonium salts and of hydrogen ion which gives the best growth of mold in

---

<sup>1</sup>The work included in this paper is taken from theses submitted by L. Weisbrodt Smith and by Helen Jo Collins in partial fulfillment of the requirements for the degree Master of Science at Iowa State College. The authors wish to express appreciation for the help received from Dr. E. I. Fulmer of the Chemistry Department, Dr. J. C. Gilman of the Botany Department, Iowa State College and from Dr. N. S. Golding of the Dairy Department, University of British Columbia, during the progress of these experiments.

Czapek's medium and furthermore, to determine the constituents necessary for the production of esterase and protease.

(1) *Adjustment of medium for mold growth.* Table 1 shows the composition of Czapek's medium, in comparison with the medium as modified by the addition of varying amounts of ammonium chloride. These media were used in the experimental work which follows.

TABLE 1. *Composition of media in grams per 1000 cc. solution*

	Czapek	Modification of Czapek
MgSO <sub>4</sub> .....	0.50.....	0.50
K <sub>2</sub> HPO <sub>4</sub> .....	1.00.....	1.00
KCl .....	0.50.....	0.50
FeSO <sub>4</sub> .....	0.01.....	0.01
NaNO <sub>3</sub> .....	2.00.....	None
NH <sub>4</sub> Cl .....	None.....	(Varying between 0.001 N and 0.4 N)
Corn sugar .....	2.50.....	2.50

Into each of a series of 50 cc. Erlenmeyer flasks was placed 15 cc. of one of the above media, the concentration of which would allow for the addition of 5 cc. of additional liquid. The flasks were plugged, sterilized at 15 pounds pressure for 12 minutes and inoculated with 5 cc. of a mold dispersion. The mold dispersion was prepared by inoculation of sterile water with loose mold from a four day agar culture. After incubating at 30° C. until the first spores appeared, the weight of dry mold felt was determined.

Several series of experiments were made, using the media described in the foregoing paragraph, in which the concentration of ammonium chloride varied from 0.001 N to 0.4 N. The results of these experiments are shown in table 2.

TABLE 2. *Weight of mold felts produced by P. roqueforti on Czapek's medium varying the concentration of NH<sub>4</sub>Cl*

Normality of NH <sub>4</sub> Cl	Grams mold felt
0.001.....	0.0012
0.010.....	0.0021
0.020.....	0.0126
0.060.....	0.0215
0.080.....	0.0301
0.100.....	0.0328
0.125.....	0.0278
0.150.....	0.0035
0.175.....	0.0024
0.200.....	0.0077
0.300.....	0.0042
0.400.....	0.0085

The varying weights of mold felt obtained indicate that there is an optimum quantity of ammonium chloride which may be used in Czapek's medium to bring about the formation of a heavier mold felt than could be

obtained in the absence of ammonium salts. The heaviest growth of mold was obtained at 0.1 N ammonium chloride, and at this normality sporulation was first noted.

It was of interest to determine the importance of iron salts in the medium. It has been suggested (3) that the organism may be able to make up certain salt deficiencies from the glass of the containing vessel. A group of experiments using Czapek's medium with 0.1 N ammonium chloride in place of sodium nitrate, in which the mold was grown in the presence and in the absence of 0.01 N ferrous sulfate, indicated that the presence of that small trace of ferrous ion fostered vigorous growth and sporulation of mold.

A study was made of the hydrogen ion concentration of the medium containing 0.1 N ammonium chloride at which the best yield of mold felt was obtained. Hydrogen ion determinations were made with the quinhydrone electrode (1) and the hydrogen ion concentration of the media adjusted under sterile conditions using the procedure described by Fulmer and Christensen (6). Media varying from pH 3 to 8 were prepared, and the weight of mold felt upon each was determined by the same procedure as previously described. The results of these experiments are shown in table 3.

TABLE 3. *Weight of mold felts produced by P. roqueforti on Czapek's medium containing 0.1 N NH<sub>4</sub>Cl and varying pH*

pH	Grams mold felt
3.00.....	0.0309
4.00.....	0.0420
4.50.....	0.0698
5.00.....	0.0526
6.00.....	0.0452
6.49.....	0.0381
7.00.....	0.0321
7.50.....	0.0269
7.80.....	0.0102
8.00.....	0.0062

It is interesting to note that the heaviest mold felt is obtained at pH 4.5, a slightly acid concentration, rather than in a neutral solution (pH 7.00) which is typical of Czapek's medium.

(2) *Adjustment of medium for the production of esterase.* The medium used for the growth of mold and formation of esterase is similar to Czapek's medium with the modifications just mentioned, and also contains ethyl butyrate to stimulate the production of esterase during formation of the mold. The medium contained, in 1000 cc. of solution, the following compounds:

MgSO <sub>4</sub> .....	0.50 grams
K <sub>2</sub> HPO <sub>4</sub> .....	1.00 "
KCl .....	0.50 "
FeSO <sub>4</sub> .....	0.01 "
NH <sub>4</sub> Cl (0.1 N) .....	5.35 "
Corn sugar .....	2.50 "
Ethyl butyrate .....	0.50 cc.



The medium was prepared, and after sterilization was adjusted to pH 4.5, as previously mentioned. Inoculation was made and the mold allowed to grow for ten days at 30° C. At this time, sporulation was well advanced. The felts were removed and both mold felt and filtrate were tested in digestion experiments for esterase activity.

(3) *Adjustment of medium for production of protease.* It was desired to add casein to the nutrient media upon which the mold was grown, in order to stimulate protease formation. Preliminary experiments indicated that, owing to the excess of nitrogen furnished by both ammonium chloride and casein, growth of mold occurred but sporulation was delayed. Since better growth was obtained on a medium in which sugar, casein, and ammonium chloride were present, than when any of these were omitted, it seemed to be necessary to obtain a suitable balance for these constituents. It was shown also, that the presence of casein brought about a change in the optimum hydrogen ion concentration for mold growth.

All mold growth experiments were carried out as described in section I (1) of this paper. Concentrations of casein varying from one-tenth per cent to five per cent were added to Czapek's medium, and the optimum growth was obtained at one per cent casein. Using casein in the medium, it was necessary to adjust the concentration of hydrogen ion and of ammonium chloride in order to obtain the best growth and sporulation of mold. When the usual amount (0.1 N) ammonium chloride was used with one per cent casein, the excess of nitrogen caused a cottony growth of mycelium with little sporulation. This condition was not desirable and was corrected by the adjustment made as shown in tables 4 and 5.

TABLE 4. *Weight of mold felt obtained by growth of P. roqueforti on modified Czapek's medium containing one per cent casein and adjusted to varying pH.*

pH	Grams mold felt
1.30.....	none
4.95.....	0.0225
5.59.....	0.0358
5.62.....	0.0323
6.32.....	0.0250
7.00.....	0.0235

TABLE 5. *Weight of mold felt obtained by growth of P. roqueforti on modified Czapek's medium containing one per cent casein, adjusted to pH 5.6 and varying the concentration of NH<sub>4</sub>Cl*

Normality of NH <sub>4</sub> Cl	Grams mold felt
0.10.....	0.0289
0.05.....	0.0316
0.03.....	0.0335
0.01.....	0.0177
0.00.....	0.0178

The weight of mold felt obtained in these experiments indicates that the best growth of mold is obtained at pH 5.6 in a medium in which one per cent casein is present.

The optimum quantity of ammonium chloride is shown to be 0.03 N, as compared with .1 N in a medium in which casein is not present and in which ammonium chloride is the only source of nitrogen. With the adjustment here indicated of casein, ammonium chloride, and hydrogen ion concentration, good growth and sporulation were obtained.

The medium most suitable for growth of mold with production of protease contained, in 1000 cc. of solution, the following compounds

MgSO <sub>4</sub> .....	0.50 grams
K <sub>2</sub> HPO <sub>4</sub> .....	1.00 "
KCl .....	0.50 "
FeSO <sub>4</sub> .....	0.01 "
NH <sub>4</sub> Cl (0.03 N) .....	1.61 "
Sugar .....	2.50 "
Casein .....	10.00 "

The medium prepared and adjusted as before mentioned, to a hydrogen ion concentration of pH 5.6, was inoculated and the mold allowed to grow for ten days at 30° C. The felts were removed, and both mold felt and filtrate were tested in digestion experiments for protease activity.

## II DIGESTION EXPERIMENTS

(1) *Preparation of enzyme.* The mold felts were removed, washed and mascerated with distilled water. This extract was allowed to stand at ice box temperature with frequent agitations, for several hours. After the solid residue had settled, the extract was used as intra-cellular enzyme. The media filtrates were always tested for extra-cellular enzyme.

(2) *Experiments to test esterase activity.* Into 50 cc. Erlenmeyer flasks each containing 3 cc. of molar disodium hydrogen phosphate, 2 cc. of conductivity water, and one-half cc. ethyl butyrate, were placed 10 cc. of the extra-cellular enzyme; into similarly prepared flasks were placed 10 cc. of the intra-cellular enzyme; the blank determinations were prepared by adding 10 cc. of boiled enzyme to flasks containing the substrate. To each flask was added 4 drops of toluene to prevent bacterial action. Digestion proceeded at 30° C. for ten days, then the contents of the flasks were titrated with standard sodium hydroxide. The difference between the amount of acid present in the flasks containing active enzyme and that in the flasks containing boiled enzyme represented the degree of hydrolysis of the ethyl butyrate, and hence the activity of the esterase.

A series of duplicate experiments, using mold grown on Czapek's medium in comparison with mold grown on the medium described in section I (2) of this paper, show that a greater enzyme activity is obtained from those intra and extra-cellular extracts prepared from media at optimum hydrogen ion and ammonium chloride concentration than in those extracts obtained from Czapek's medium. The results of these experiments also support the hypothesis suggested by Dox (4) that when mold felts are removed after sporulation is well advanced, the greatest enzyme action is found in the mold filtrate which has been termed extra-cellular enzyme. Table 6 summarizes the results obtained in these experiments.

TABLE 6. Amount of esterase (expressed in cc. of 0.0847 N NaOH) produced by *P. roqueforti* on Czapek's medium with various modifications

Media	cc. of base for	
	Mold extract	Mold filtrate
Czapek's medium as used by Dox .....	0.161.....	0.242
Czapek's medium with 0.10 N $\text{NH}_4\text{Cl}$ .....	0.200.....	0.990
Czapek's medium with 0.10 N $\text{NH}_4\text{Cl}$ , pH 4.5 .....	0.200.....	1.550

(3) *Experiments to test protease activity.* The substrate was prepared by dissolving casein in dilute hydrochloric acid or in dilute sodium hydroxide (10) and the mixture brought to the desired hydrogen ion concentration by titration with standard base or acid. Hydrogen ion concentration was measured by the quinhydrone electrode method (1). Enzyme was added to the substrate, four drops of toluene to prevent bacterial action, and the mixture incubated at 30° C. for 48 hours. Undigested casein was precipitated by the addition of 20 per cent sodium sulfate to those mixtures more acid than pH 7.00 or by addition of 10 per cent acetic acid and then sodium sulfate to those mixtures more alkaline than pH 7.00. The nitrogen of undigested casein was determined by the Kjeldahl method and calculations made on the basis of blanks containing boiled enzyme dispersion.

The extracts prepared from synthetic media at optimum ammonium chloride and hydrogen ion concentration showed proteolytic activity comparable with those extracts prepared from mold grown on separated milk. The use of synthetic media rather than milk for the preparation of enzyme material makes it possible to add to a digestion substrate, enzyme extract of less uncertain composition and simplifies the problem of extraction of enzyme and of studying optimum digestive conditions.

TABLE 7. Influence of hydrogen ion concentration upon digestion of 0.5 gm. casein by protease of *P. roqueforti*

pH	Grams casein recovered	Grams casein recovered (boiled enzyme)
3.37.....	0.421.....	0.418
3.50.....	0.414.....	0.414
5.29.....	0.315.....	0.400
5.90.....	0.380.....	0.406
6.45.....	0.412.....	0.408
7.90.....	0.412.....	0.408
8.90.....	0.409.....	0.424

In order to determine the optimum hydrogen ion concentration of the digestion substrate, proteolytic enzyme was allowed to act upon casein mixtures varying from pH 3.37 to 8.90. After 48 hours at 30° C., the amount of undigested casein was determined as previously described. The results of these experiments are given in table 7. Hydrogen ion concentration, pH 5.30, was determined as optimum for proteolytic digestion. It is significant that the hydrogen ion concentration optimum for digestion of casein by protease of *P. roqueforti* corresponds closely to the hydrogen ion concentration of the medium at pH 5.6 determined as optimum for growth of the mold in a casein media.

## SUMMARY

These experiments have determined the constituents of a synthetic medium which gives an increase in the weight of mold over that obtained in comparative experiments by use of Czapek's solution. Moreover they have given evidence that the increase in the weight of mold fosters an increase of esterase and protease activity in the extracts. The optimum hydrogen ion concentration for proteolytic digestion at 30° C. has been determined at pH 5.30.

## LITERATURE CITED

- (1) CLARK AND COLLINS  
1927. Soil Science. 24:453.
- (2) CURRIE  
1914. Jour. Agr. Res. 2:1.
- (3) DOX  
1909. Jour. Biol. Chem. No. 5. 6:461.
- (4) DOX  
1910. U. S. D. A. Bureau An. Ind. Bull. 120.
- (5) FULMER  
1925. Colloid Symposium Monograph II, 204.
- (6) FULMER AND CHRISTENSEN  
1925. Jour Ind. Eng. Chem. 17:935, No. 9.
- (7) FULMER, SHEERWOOD AND NELSON  
1921. J. A. C. S. 43:191.  
1924. Jour. Ind. Eng. Chem. 16:921.
- (8) GOLDING  
1924. Trans. Roy. Soc. Canada. 18:67.
- (9) SAITO  
1910. Wochenschrift für Brauerei. 27:181.
- (10) SHERMAN AND NEUN  
1916. J. A. C. S. 38:2200.
- (11) THOM  
1914. Conn. (Storrs) Exp. Sta. Bull. 79.





# INDEX

## Author Index

Becker, Elery R., 215.  
 Brown, P. E., 325, 425.  
 Brown, R. E., 355.  
 Buchanan, J. H., 331, 359, 431, 441, 451.  
 Chapman, O. W., 441.  
 Collins, Helen Jo, 485.  
 Drake, C. J., 253.  
 Emmerson, M. A., 215.  
 Fothergill, Robert E., 351.  
 Fulmer, Ellis I., 1.  
 Gilman, Henry, 27, 35, 323, 351, 355.  
 Harris, W. D., 37.  
 Hendrickson, George O., 49.  
 Hewlett, Amiot P., 27, 355.  
 Hsiung, Ta-Shih, 359.  
 Jones, R. M., 253.  
 Lambert, W. V., 343.  
 Leermakers, J. A., 323.  
 LeVesconte, Amy, 331, 451.  
 Levine, Max, 331, 451.  
 Moore, Edmond E., 1.  
 Moore, Marjorie B., 431.  
 Muncie, J. H., 263, 315.  
 Naylor, N. M., 485.  
 Pospishul, B. K., 37.  
 Schott, R. G., 343.  
 Schulz, J. A., 215.  
 Smith, F. B., 325.  
 Smith, L. Weisbrodt, 485.  
 Suit, Ross F., 263.  
 Van Puersen, R. L., 37.  
 Walker, R. H., 425.  
 Werkman, C. H., 459.  
 Wilster, G., 181.  
 Wright, George F., 35, 355.

## Subject Index

Acetic acid, 1, 2, 3, 5, 8, 21, 22, 462, 463.  
     anhydride, 35, 36.  
 Acid, acetic, 1, 2, 3, 5, 8, 21, 22.  
     boric, 37, 38.  
     chromic, 2.  
     distillation of, 3.  
     formic, 1, 2, 3, 5, 8, 21, 22.  
     hydrochloric, 38.  
 Acidity of soils, 425.  
 Acids, changing proportions of, 463.  
     distribution of, 459.  
     non-volatile, 459, 463.  
     organic, 463.  
     volatile, 459, 463.  
*Aerobacter levans*, 451, 454, 455.  
 Agar, crystal violet bile, 270.  
 Alkali forming bacteria, 190, 191, 196,  
     197.  
     specific gravity of, 196.  
*Allantosoma brevicorniger* 363, 409.  
     *dicorniger*, 363, 409.  
     *intestinalis*, 363, 409, 411.  
 Allantosoma, key to species, 408.  
*Alloisoma trizona*, 363, 378.  
 Amoebida, 365.  
*Ampullacula ampulla*, 380.  
 Amylophosphoric acid, 441.  
*Andropogon* associes, 53.  
*Andropogon furcatus*—*Sorghastrum nut-*  
     *ans* associes, 53, 169.  
*Andropogon furcatus*—consocies, 54, 170,  
     173.  
*Andropogon furcatus*—*Spartina Mich-*  
     *auxiana* associes, 54.  
*Andropogon scoparius*—*Bouteloua curti-*  
     *pendula* association, 167, 173.  
 Anti-oxime of perilla aldehyde, 27, 28.  
 Antiseptic hypochlorite, by electrolysis,  
     37.

- Aqueous phase, 460, 461.
- Association
- Andropogon scoparius*—*Bouteloua curtipendula*, 53, 167.
  - Stipa spartea*—*Andropogon scoparius*, 53.
  - Stipa*-*Bouteloua*, 55.
  - Stipa*-*Koeleria*, 53.
  - Bouteloua hirsuta*—*B. curtipendula*, 55, 166.
- Associates
- Andropogon*, 53.
  - Andropogon furcatus*—*Spartina michauxiana* associates, 54.
  - Andropogon furcatus* — *Sorghastrum nutans*, 53.
- Azotobacter tests, 425.
- Azotobacter chroococcum*, 425.
- Bacillus radiobacter*, 284, 285, 287, 297.
- Bacteria, 227.
- types in clarified and unclarified milk, 188, 191.
  - effect in clarification on types of, 189.
  - in relation to quality of cheese, 206, 208.
- Balantidium, 361.
- B. coli*, 392.
- Balkite rectifying unit, 46.
- Barium, 38.
- Bingham plastometer, 442.
- Biological method determining sugar, 457.
- Biological estimation of glucose, 451.
- Blepharocorys, 228.
- Blepharocodon, 361.
- B. appendiculatus*, 380.
- Blepharoconus benbrookii*, 363, 377.
- B. cervicalis*, 363, 376.
- Blepharocorys, 360, 361.
- B. angusta*, 362, 363, 390, 410.
  - cardionucleata*, 363, 390.
  - curvigula*, 362, 363, 389, 410.
  - jubata*, 362, 363, 388, 410.
  - uncinata*, 363, 385.
  - valvata*, 363, 387.
  - key to species, 384.
- Blepharocordiae, key to genera, 384.
- Blepharosphaera, 361.
- B. ellipsoidalis*, 363.
  - intestinalis*, 363, 373, 374.
- Blepharoposthium, 372.
- B. pircum*, 363, 372.
- Blepharozoum zonatum*, 375.
- Bouteloua hirsuta*—*B. curtipendula* association, 55, 166.
- Boric acid, 37, 38.
- Brom phenol blue, 436.
- Bromine, reduction of, 2.
- Broom-root, 268.
- Buetschli, 220, 228, 361.
- Buetschliidae, key to genera, 369.
- Bundleia, 362.
- Bundleia postciliata*, 363, 377, 410.
- Butyric-acetic, 460, 461.
- Butyric, acid, 462.
- Butyric-propionic, 460, 461.
- Callimastigidae, 367.
- Callimastix equi*, 363, 367.
- frontalis*, 227.
- Callus knots, 270.
- Cane sugar, 27, 32.
- Carbon dioxide, 438, 439.
- pH of solutions, 435.
  - as germicidal agent, 431.
- Carbon monoxide, measurement, 3.
- Carbonated beverages, 439.
- Carex socias, 54, 172.
- Carrington loam, 427.
- Cellulose, digestion of, 217, 218.
- Centrifugal force, effect of, 195, 196.
- Charon, 228.
- Charon equi*, 361, 363, 391, 411.
- Charon, key to species, 391.
- Charon ventriculi*, 391.
- Cheese
- bacteria in, 206, 207, 208.
  - cheddar, 181.
  - quality of, 207, 208.
  - scores on, 202, 203, 205.
- Chemical analysis, 230.
- Chloride dihydrate, stannous, 36.
- Chlor-organic compounds, 37.
- Chlorine, 37, 38.
- Chromic acid, reduction of, 2.
- Chrysanthemum frutescens*, 283, 284, 294, 298.
- Ciliata, 411.
- key to orders, 368.
- Ciliates, 359, 360, 362.
- Ciliophora, 368.
- Clarification, effect of, on bacteria in milk, 184, 185, 186, 187, 193, 194.
- influence of, 187, 198, 206, 208.
  - effect of, determined by plate count, methylene blue test and the fermentation test, 193, 194.
- Clarification of milk for American cheddar cheese, 181.
- Clostridium pasteurianum*, 425.
- Coagulators, acid, 189, 191.
- Cochliotrox periachtum*, 363, 407.
- Coleoptra, 91, 167, 168, 169, 170, 171, 172, 173, 174.
- Collembola, order, 57.
- Colorimetric determinations, 435, 436, 438, 439.
- Columba livia*, 254.
- Commensals, 215.
- harmless, 217.
- Concentration, 463.
- Conductivity measurements, 432, 439.
- Conductometric titration, 5.
- Consocias
- Spartina*, 54, 56, 171, 173.
  - Andropogon furcatus*, 54.

- Crowngall of *Rumex* and *Rheum*, 315.  
 Crowngall, studies on, 263.  
   prevalence of, 263, 264.  
   infection experiments, 292.  
 Cycloposthiidae, key to genera, 393.  
 Cycloposthium, 360, 362.  
*Cycloposthium affinae*, 363, 398.  
   *bipalmatum*, 362, 363, 394, 410.  
   *corrugatum*, 362, 363, 399.  
   *dentiferum*, 363, 395.  
   *edentatum*, 363, 396.  
   *scutigerum*, 363, 397, 410.  
   key to species, 393.  
 Czapek's medium, 466, 469.  
   modification of, 466.  
 Dakin solution, 37, 38, 45, 46, 47.  
 Dasytricha, 220, 228.  
 Derris powder, use of, 256.  
 Desmidis, 361.  
 Determination of organic acids, 459.  
 Didesmis, key to species, 370.  
*Didesmis ovalis*, 363, 370, 410.  
   *quadrata*, 363, 371, 410.  
   *spiralis*, 363, 371.  
 Digestion experiments, 469, 471.  
 Diplocladium, 220, 228, 360.  
   *caudatum hamatum*, 229.  
   *bursa*, 228.  
   *multi-vesiculatum*, 228.  
 Diptera, 125, 167, 168, 169, 170, 172, 173.  
 Distillation of acids, 3.  
   separation by, 3.  
   steam, 3.  
 Distomatidae, 367.  
*Ditoxum funinucleum*, 363, 408, 411.  
 Duclaux, 459, 463.  
 Dulcin, 27, 33.  
 Eau de Javelle, 37.  
   Labarrique, 37.  
 Electrolysis, 38.  
 Electrolytic cell, 38.  
 Endamoeba, 361.  
   *E. equi*, 366.  
   *gedoelsti*, 363, 365.  
 Entodinium, 220, 228, 360.  
   *E. caudatum*, 229.  
   *simplex*, 229.  
   *minimum*, 229.  
   *Longinucleatum*, 229.  
   *vorax*, 229.  
 Enzymes  
   action, 465.  
   lipolytic, 465.  
   preparation of, 469.  
   proteolytic, 465.  
 Ephemerida, order, 64.  
 Esterase, 465.  
   experiments to test activity of, 469.  
   production of, 467.  
   amount produced by *P. roqueforti*.  
 Esterase and protease of *Penicillium roqueforti*, 465.  
 Ethyl ether, 460, 461, 462.  
   water partition, 459.  
 Fatty acids, 459, 460, 463.  
   quantitative determinations of, 463.  
 Fauna, 359, 360.  
   protozoan, 215.  
   infusorian, 228.  
 Ferrous iron, 467.  
   sulfate, 467.  
 Formic acid, quantitative determination of, 2.  
 Fructose media, pH change of, 455.  
 Furan series, correlation of constitution, 27.  
 Furfural, 35, 36.  
   amine, 28.  
   condensation of, 27.  
   urea, 28.  
   diacetate, preparation of, 35, 36.  
 Furfuraldoxime, 28.  
 Furoic sulfide, 27.  
 Furonitrile, 27, 32, 33.  
 Furylacrolein, oxime of, 31.  
 Furylacrylamide, 31.  
 Furylacryloyl chloride, 31.  
 Furylacrylonitrile, 27, 31, 33.  
 Furylalanine, 27.  
 Furyl-allylamine, 31.  
 Furyl-p-bromophenyl-methylurea, 30.  
   ketone, 30.  
   oxime of, 31.  
 Furyl chloride, 29.  
 Furyl phenyl  
   ketone, 29.  
   methylamine, 29.  
   methylurea, 29.  
   oxime of, 29.  
 Furyl-p-phenetyl ketone, 30.  
   oxime of, 30.  
 Furyl-p-phenetyl-methylamine, 30.  
 Furyl-p-phenetyl-methylurea, 30.  
 Gelatinization temperature, 441.  
 Giardia, 361.  
   *G. equi*, 368.  
 Glucose, concentration of, 451, 456.  
   decomposition of, 451.  
   media, pH change of, 455.  
 Grassland climax, 53.  
 Grundy silt loam, 426, 428.  
*Haemoproteus columbae* Celli and Sanfelice, 254.  
 Hairyroot, on apple nursery stock, 263.  
   on quince cuttings, 276.  
   on apple seedlings, 277.  
   morphology of, 284.  
   cultural studies of, 284.  
   cultural characters, 287.  
   infection experiments, 292.  
 Halteridium, 254.  
 Hemiptera, 65, 166, 167, 168, 169, 170, 171, 172, 173, 174.  
 Heterotrichida, 392.



- Hippoboscidae, 254.  
 Holophryoides ,361, 374.  
     *H. ovalis*, 374.  
 Holotrichida, 368.  
 Homoptera, 77, 166, 167, 168, 169, 170,  
     171, 172, 173, 174.  
 Host-parasite relationships, 215.  
 Hydrochloric acid, 38.  
 Hydrogen, 38, 45.  
 Hydrolysis of amylopectin, 441, 442.  
 Hymenoptera, 148, 167, 168, 169, 170,  
     172, 173.  
 Hypochlorite, 38.  
     antiseptic, 37.  
     sodium, 37, 48.  
 Hydrocarbons, 438.  
 Hydrogen electrode, 437.  
 Hydrogen ion concentration  
     calculation of, 432.  
 Hydrogen ion concentration of neutral  
     water, 431.  
 Immiscible solvents, 459.  
 Infusoria of mammals, 359.  
 Infusoriacidal agent, 220.  
 Insect fauna of Iowa prairies, 49.  
 Inversion constants of sucrose, 454, 455,  
     456, 457.  
 Iodates, reduction of, 2.  
 Ionization constant, 431, 439.  
     calculation of, 432, 439, 442.  
 Iron salts, 467.  
 Isolations, from overgrowths, 279.  
     burr-knot of hairyroot, 282.  
     fibrous hairyroot, 281.  
     from hairy-knots, 279.  
     seedlings, 280.  
 Isolation studies upon overgrowths and  
     hairyroot, 266.  
     budded quince trees, 275.  
     methods, 267.  
     on apple seedlings, 279.  
     of fibrous hairyroot, 271.  
     of burr-knot hairyroot, 272.  
 Isopropyl ether, 459.  
 Isotonic solution, 47, 48.  
 Isotricha, 220, 228.  
     *I. intestinalis*, 229.  
 Knotted piece-root grafted apple trees,  
     266.  
 Kohlrausch bridge, 11.  
 Lactic-acetic, 460.  
     butyric, 460.  
 Larva, pigeon fly, 255.  
 Lepidoptera, 120, 167, 168, 169, 170, 171,  
     172, 173.  
 Lime deficiencies in soil, 425.  
     treatment, 426, 427.  
 Malaria, pigeon, 253, 254.  
 Mastigophora, 366, 411.  
 Mechanical services, 219.  
 Medium, composition of, 465.  
     studies on, 465.  
 Mercuric salts, reduction of, 2.  
 Metazoan host, 215.  
 Metazoan invertebrates, 362.  
 Metallic magnesium, reduction by, 3.  
 Micro-analytical methods, 3.  
 Milk, pasteurization of, 181.  
     effect of clarification of, 184.  
     clarified, 188, 198.  
     unclarified, 198, 202.  
 Mold felt, weight of, 466, 471.  
 Monadidae, 366.  
 Monograph on the protozoa of the large  
     intestine of the horse, 359.  
 Neuroptera, order, 63.  
 Nichrome electrodes, 39.  
 Nicotine sulfate, 256.  
 Nitro-alcohols of furan series, 27.  
 Nitrogen-fixing bacteria, 426.  
 Nitrogen fixation, 425.  
 Nitrogen-free mannite solution, 426.  
 Odonata, order, 64.  
*Oikomonas equi*, 363, 366.  
 Oligotrichs, 411.  
 Omega-furyl-allyl-urea, 31.  
 Ophryoscolex, 220, 228.  
 Order  
     Coleoptera, 91, 167, 168, 169, 170, 171,  
         172, 173, 174.  
     Collembola, 57.  
     Diptera, 125, 167, 168, 169, 170, 172,  
         173.  
     Ephemera, 64.  
     Hemiptera, 56, 166, 167, 168, 169, 170,  
         171, 172, 173, 174.  
     Homoptera, 77, 166, 167, 168, 169, 170,  
         171, 172, 173, 174.  
     Hymenoptera, 148, 167, 168, 169, 170,  
         172, 173.  
     Lepidoptera, 120, 167, 168, 169, 170,  
         171, 172, 173.  
     Neuroptera, 63.  
     Odonata, 64.  
     Orthoptera, 58, 166, 167, 168, 169, 170,  
         171, 173.  
     Plecoptera, 65.  
     Thysanoptera, 65.  
     Trichoptera, 120.  
 Orthoptera, 58, 166, 167, 168, 169, 170;  
     171, 173.  
 Overgrowths of nursery stock, 263, 264.  
 Overgrowths, location of, 266.  
     wooly knot, 270.  
 Oximes, 27.  
 Paraisotricha, 360.  
     *P. beckeri*, 363, 383.  
     *colpoidea*, 363, 381, 411.  
     *minuta*, 363, 383.  
     key to species, 381.  
 Parasites, 215.  
     injurious, 217.  
 Parasite, specialized, 254.  
 Paris daisy, crown gall of, 263.

- Partition coefficient, 463.  
 constant, 460, 461.  
*Penicillium roqueforti*, 465.  
 Peptonizers, acid, 189, 190.  
 neutral, 189, 190, 191.  
 Perilla aldehyde, 27.  
 nitrite, 27.  
 Phenolphthalein, 38.  
 Phenyl-furymethyl urea, 29.  
 Phosphorus deficiencies in soil, 425.  
 Pigeon fly and pigeon malaria in Iowa,  
 253.  
 control of, 256.  
 Platinum chloride, reduction by, 2.  
 Plant substances, conversion of, 216.  
 Plasmadroma, 365.  
 Plecoptera, order, 65.  
*Polygonum amphibium* socies, 55, 172,  
 174.  
 Polymastigina, 367.  
*Polymorpha ampulla*, 363, 379.  
 Potassium permanganate, reduction of, 2.  
 Potentiometric method, 437.  
 Prairie, 53.  
 true, 53.  
 sub-climax, 53.  
 mixed, 55.  
 studies of insect fauna of Iowa, 49.  
 Properties of starch with relation to time  
 of formation of starch gels, 441.  
 Propionic-acetic, 460, 461.  
 Propionic acid, 462, 463.  
*Prorodonopsis coli*, 375.  
*composita*, 375.  
 Protease, 465.  
 digestion of casein by, 470.  
 experiments to test activity, 470.  
 production of, 468.  
 Protomonadina, 366.  
 Protozoa, 215, 216.  
 entozoic, 215.  
 effect on pH, putrefaction, 233.  
 method of studying living, 362.  
 staining, 362.  
*Pseudolynchia maura* (Bigot), 253, 254.  
*Pseudomonas tumefaciens*, Sm. and Town,  
 263.  
 hosts of, 270.  
 Puparia, pigeon fly, 255.  
 Pyrethrum, for control of pigeon flies, 256.  
 powder, 256.  
 soap, 256.  
 Quinhydrone electrode, 467.  
*Rheum rhaponticum* L., 315.  
 Rhizopoda, 365, 411.  
 Roquefort cheese, 465.  
*Rumex crispus* L., 315.  
 Ruminants, domestic, 215.  
 protein requirements of, 216.  
 Rumen infusoria, 215.  
 non-infusorian life of, 227.  
 Saccharin, 27, 33.  
 Schizomycetes, 215.  
 reduction of, 217.  
 Silica gel medium, 426, 427.  
 Spartina consocios, 54, 171, 173.  
 Socies  
 Carex, 54, 172.  
*Polygonum amphibium*, 55.  
 Sodium hypochlorite, 37, 47, 48.  
 bicarbonate, 37.  
 chloride, 38, 47, 48.  
 chromate, 38.  
*Spirodinium equi*, 363, 400, 411.  
 Standards for colorimetric measurements  
 of pH, 437.  
 Starch gels, 441.  
 Starch suspensions, 442.  
 effect of concentration and tempera-  
 ture upon, 443.  
 Steam distillation, 3.  
 Stipa-Bouteloua formation, 53.  
 association, 55.  
 Stipa-Koeleria association, 53.  
 Stipa spartea—*Andropogon scoparius* as-  
 sociation, 168, 173.  
*Streptococcus lactis*, 189.  
 Stomatea, key to sections, 368.  
 Suetoria, 362, 411.  
 Stareh, 441.  
 Studies of crowngall, overgrowths and  
 hairyroot on apple nursery stock, 263.  
 Studies on the insect fauna of Iowa  
 prairies, 49.  
 Studies on nitrogen fixation in some Iowa  
 soils, 425.  
 Sucrose, inversion of, 452.  
 Sulfuric acid, 36.  
 decomposition by, 3.  
 Symbionts, 215.  
 Syneresis, 441, 442, 443, 447, 449.  
 effect of salts on, 448, 449.  
 of starch gels, 446, 447, 448.  
 Sweetnes, methods of testing, 32, 33.  
 Tantalum electrodes, used as cathodes,  
 39.  
 Tetratoxum, 361.  
 key to species, 403.  
*T. excavatum*, 363, 404.  
*parvum*, 363, 405.  
*unifasciculatum*, 363, 403, 411.  
 Tysanoptera, order, 65.  
 Titrations of carbonates, 434.  
 Titration, conductometric, 1.  
*Torula cremoris*, 196, 197, 210.  
 Trees, apple, budded and grafted, 264,  
 265.  
 Trichomonadidae, 367.  
*Triadinium caudatum*, 363, 400, 410.  
 galea, 363, 401, 410.  
 key to species, 400.  
*minimum*, 363, 402, 410.  
*Trichomonas*, 361.  
*T. equi*, 363, 367.



- Trichostomata, key to families, 381.  
*Trichomonas ruminantium*, 227.  
Trichoptera, order, 120.  
*Tripalmaria dogieli*, 363, 406, 410.  
Tri-sodium phosphate, 446.  
Trophozoites, 229, 411.  
*Trypanosoma hunnai* Pitagula, 254.  
Variation of hydrogen ion concentration, 431.  
Viscosimeters, 442.  
    Doolittle, 442, 443, 444, 445.  
    MacMichael, 442, 443.  
    Ostwald, 442, 443, 444, 445.  
Redwood, 442.  
Scott, 442.  
Stormer, 442.  
Torsion, 442.  
Viscosimetric methods, 443.  
Viscosity, 441.  
    decrease in, 443, 446.  
    increase in, 446.  
    of starch, 443, 444.  
    of starch suspensions, effect of added reagents upon, 445.  
Yeasts, specific gravity, 196.  
Zoomastigma, 366.  
Zymologist, 459, 463.